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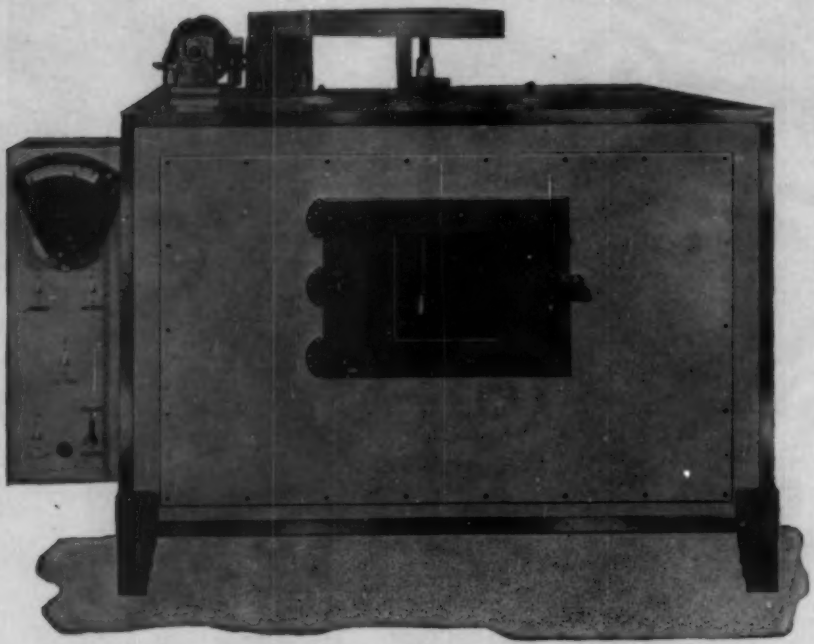

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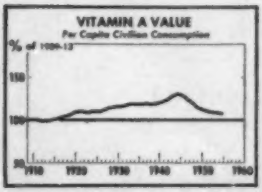

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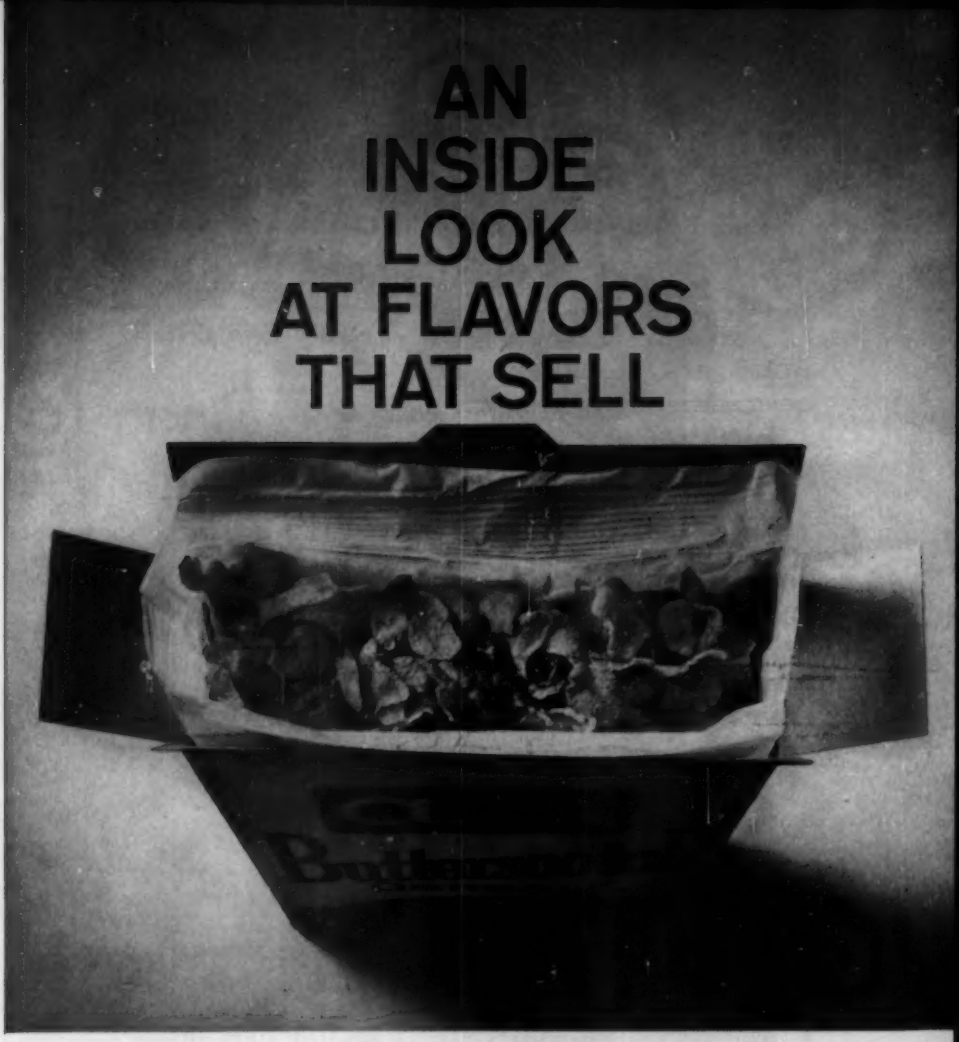

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CEREAL CHEMISTRY

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THE BROMATE REACTION IN DOUGH

IV. Effect of Reducing Agents[1]

W. BUSHUK AND I. HLYNKA

ABSTRACT

The rate of bromate ion reaction in dough is increased by thioglycolic acid, L(+) cysteine hydrochloride, glutathione (reduced), thiolated gelatin, sodium bisulfite, and sodium borohydride if these reagents are incorporated into the dough before addition of potassium bromate. For each reagent, the increase in the reaction rate is proportional to the amount added. Increases in the rate are similar for the first four reagents, slightly lower for bisulfite, and much lower for borohydride. These results are consistent with the hypothesis that the bromate ion reacts principally with –SH groups and that the number available is increased by the reagents studied.

Reducing agents, such as bisulfite and thioglycolate, are known to split disulfide linkages in proteins to produce one or sometimes two sulfhydryl groups (8); this reaction is considered to be responsible for the changes in the physical properties of dough caused by reducing agents (5,7,9). Since bromate ions react principally with sulfhydryl groups in dough, the rate of this reaction should be increased by prior addition of reducing agents. Quantitative results consistent with these postulates are reported in the present paper, which continues a larger study seeking information on various aspects of the bromate reaction in dough (1,2,3).

Materials and Methods

The flour used in this study was an untreated, straight-grade flour commercially milled from hard red spring wheat. Its protein and ash contents were 13.3 and 0.46% on a 14% moisture basis. The reducing agents used and their grade or origin were as follows:

[1] Manuscript received December 27, 1960. Paper No. 197 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Canada. Presented at the Western Regional Conference of The Chemical Institute of Canada, Regina, Canada, September 1960.

Reagent	*Grade or Origin*
Sodium bisulfite	Reagent
Sodium borohydride	98+%; Metal Hydrides Inc.
Thioglycolic acid	80% in water; analytical
L(+) Cysteine hydrochloride	General Biochemicals Corp.
Glutathione (reduced)	Nutritional Biochemicals, Inc.
Thiolated gelatin	Schwarz Thiogel A.

Ordinary gelatin, used in control experiments on thiolated gelatin, was the Knox unflavored type.

The normal method for preparing doughs was modified to obviate the rapid reaction of the reducing agent with bromate ions when they are added to the water used to prepare the dough. This was accomplished by two-stage mixing. The flour was first mixed for 2.5 minutes with 90% of the water containing the reducing agent. The remaining 10% of the water, containing the required amount of potassium bromate, was then added and mixing was continued for another 2.5 minutes. All doughs were mixed under nitrogen to 60% absorption.

Residual bromate ion in dough was determined by the method described in the first paper of this series (1). The initial potassium bromate concentration used in this study was 18.75 mg. per kg. of dough (30 p.p.m. of flour).

Results and Discussion

Preliminary Experiments. In studies seeking to determine the effect of a reducing agent on the reaction of an oxidizing agent with a third substance, it is difficult to design unambiguous experiments. Because of the rapid reaction of most of the reducing agents used with bromate ions in aqueous solution, the two substances could not be added to the flour simultaneously and the dough mixed in the usual manner. Two-stage mixing was designed to overcome this difficulty. The development of stickiness in the doughs with all reagents except thiolated gelatin (see below) after the first 2.5 minutes of mixing was taken as evidence of a definite reaction between the reducing agent and flour proteins. Furthermore, aqueous extracts of the doughs prepared immediately after the first stage of mixing did not show any residual reducing agent for the highest concentrations used. Unreacted reducing agent was extracted from doughs which contained approximately two times the highest concentration of reducing agent (except thiolated gelatin) used in this study. In another type of experiment, the dough was allowed to rest in the mixer bowl for 7.5 minutes after the first 2.5 minutes of mixing and prior to the addition of the bromate. The rate of bromate disappearance in this dough was the same as that in the dough to which bromate was added im-

mediately after the initial 2.5 minutes of mixing. Accordingly, on the basis of this evidence, it was concluded that all of the originally added reducing agent reacts in the dough in the 2.5 minutes of mixing. However, these results do not completely rule out the possibility that some reducing agent remains in the dough in an adsorbed, unextractable form and might thus be available for direct reaction with bromate ions.

Effect of Reducing Agents on the Bromate Reaction. Figure 1 shows the bromate reactions in doughs which were initially treated with various amounts of sodium bisufite. The rate of bromate reaction in the bisulfite-treated doughs increased with increasing concentration of reducing agent. When the addition of bisulfite (1 μmole per g.) and bromate to the two-stage dough was reversed, the amount of bromate that reacted at each reaction time in this dough was about 15% higher than that shown in Fig. 1 for the same concentration of bisulfite. This 15% increase can be attributed to a small amount of direct reaction of bisulfite with bromate ion which probably occurs along with the reaction of bisulfite with protein disulfide.

Results (Fig. 1) show that the reaction of bromate ion in dough increases with increasing amount of bisulfite initially incorporated into the dough. It has been postulated that bisulfite reacts with disulfide groups of wheat proteins to produce one sulfhydryl and one S-sulfonate (8); accordingly, it is concluded that the increase in the bromate reaction is the direct result of the increase in the number

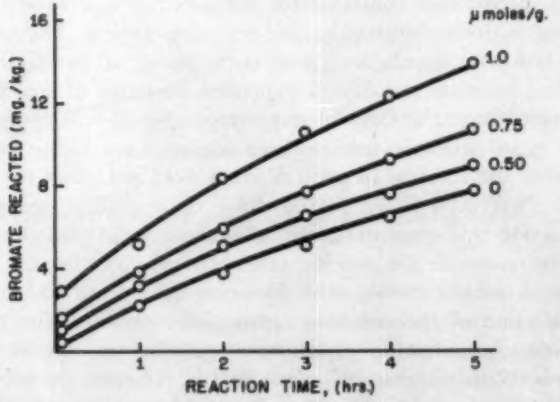

Fig. 1. Reaction of bromate in doughs treated with various amounts of sodium bisulfite. Initial potassium bromate concentration was 18.75 mg. per kg. Bisulfite concentrations given in μmoles per g. of dough.

of sulfhydryls available for reaction. These results can therefore be cited as additional evidence consistent with the hypothesis that bromate ion reacts in dough with sulfhydryl groups.

Figure 2 gives first-order plots for the data of Fig. 1. The data give good straight lines; accordingly, these reactions can be classified as first-order. The slopes of the first-order lines increased with increasing concentration of reducing agent, and this makes possible the use of the specific rate constant, which is directly related to the slope, as an index of the effect of the reducing agent.

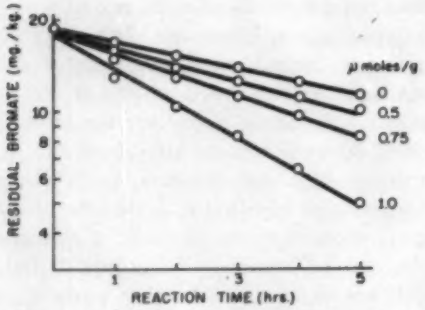

Fig. 2. First-order plots for the reactions shown in Fig. 1.

Results analogous to those obtained with bisulfite were also obtained with the other reducing agents used in this study. Table I gives the specific rate constants for the bromate reactions in doughs containing various amounts of the reducing agents. The concentrations of reducing agents are given on a per g. of dough basis, and the rate of bromate reaction is expressed in terms of the first-order specific rate constant. Control experiment for thiolated gelatin, in which 5 g. of ordinary gelatin were included in 320 g. of dough, showed that the increase in protein alone does not affect the bromate reaction. That is, it is the introduction of sulfhydryl groups *per se* or by reaction that gives the increase in the bromate reaction.

The increases in the specific rate constant (Δk) for the bromate reactions in doughs treated with reducing agents are plotted in Fig. 3 as a function of the reducing agent concentration. The point for the highest concentration of thiolated gelatin was not included in this graph. With all reagents, considerable increases in the bromate reaction were obtained. The change in the rate constant increased approximately linearly with concentration. The slight deviation from linearity for some of the reagents is not considered as highly significant.

TABLE I

FIRST-ORDER SPECIFIC RATE CONSTANTS AT INCREASING CONCENTRATIONS OF VARIOUS REDUCING AGENTS

(Potassium bromate concentration = 18.75 mg/kg of dough)

Concentration	$k \times 10^5$			
	Bisulfite	Thioglycolate	Glutathione	Cysteine
μmoles/g	*sec⁻¹*	*sec⁻¹*	*sec⁻¹*	*sec⁻¹*
0	2.82	2.82	2.82	2.82
0.25	..	4.45	4.79	5.24
0.50	3.56	7.57	9.05	9.57
0.75	4.38	...	...	...
1.0	7.15	14.82	14.03	16.57

Thiolated Gelatin			Sodium Borohydride	
Concentration		$k \times 10^5$	Concentration	$k \times 10^5$
mg/g	*μmoles SH/g*[a]	*sec⁻¹*	*μmoles/g*	*sec⁻¹*
0	0	2.82	0	2.82
0.78	0.125	4.40	4.13	5.31
1.56	0.25	6.21	8.26	6.86
3.10	0.50	8.95	16.52	10.97
7.80	1.25	17.05		
15.60	2.5	26.10		

[a] Calculated on the basis of –SH content of 16 per 100,000 molecular weight.

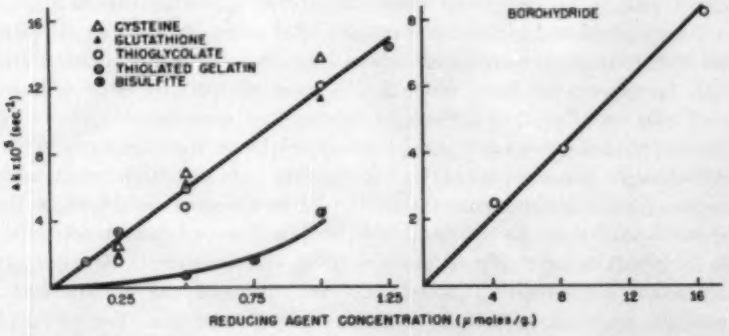

Fig. 3. The increase in the first-order rate constant for bromate reaction in doughs treated with increasing amounts of various reducing agents, plotted as a function of reducing agent concentration (per g. of dough).

Effect of Reducing Agents on Handling Properties of Dough. This study presented an opportunity to examine the effect of the various reducing agents used on the handling properties of dough, and, although the observations that were made are qualitative, it seemed desirable to put them on record.

All the reducing agents used except thiolated gelatin affected the physical properties of dough to such an extent that it was difficult

to handle them in the usual manner. The doughs were slack, sticky, and highly extensible. As the bromate reaction proceeded, a definite decrease in stickiness was observed; however, it was not entirely overcome even after a reaction time of 5 hours. This suggests that after 5 hours the sulfhydryl content has not yet decreased to the original level in the unreduced dough.

This conclusion can be supported by calculating the amount of sulfhydryl remaining in a dough treated with cysteine at the rate of 1.6 μmole per g. of flour. If it is assumed that the original flour had a sulfhydryl content of about 1 μmole per g. (10) and that all the cysteine reacts with disulfide groups to produce two sulfhydryls, i.e., 1.6 μmole per g., the total sulfhydryl content is of the order of 2.6 μmole. During 5 hours, the loss of bromate in such a dough was 28 p.p.m. of flour, which is equivalent to 1.01 μmole of sulfhydryl per g. of flour. Thus, after 5 hours, the dough would still contain 1.6 μmole of sulfhydryl as compared to an original content of about 1 μmole.

The effect of thiolated gelatin on the physical properties of dough is significantly different from that produced by the other reducing agents and deserves special comment. Doughs containing as much as 5 g. of thiolated gelatin (2.5 μmoles –SH per g. of dough) did not feel different from control doughs. Two hypothetical explanations, both based on the high molecular weight of the thiolated gelatin used, can be offered at present to explain the absence of a reducing effect of this reagent on dough. First, it might be that the rate of the interchange reaction is negligible because of the high molecular weights of the components involved; the increase in the bromate reaction would then be the result of the reaction of bromate ion with the sulfhydryls of thiolated gelatin. This latter reaction, in aqueous solution of neutral pH, proceeds at about the same rate as the normal bromate reaction in dough. Second, it might be that the physical properties of the protein that results from the interchange are similar to the properties of the original protein, because the relative size of the molecules produced is not very different from the original size.

General Discussion

The main difference between the effects of the various reducing agents shown in Fig. 3 is in the magnitude of the increase in the bromate reaction per equivalent weight of each reagent. Thioglycolate, cysteine, and glutathione have approximately the same effect (thiolated gelatin is not included in this comparison for reasons given below). This is slightly higher than the effect of bisulfite. The small

difference between the effects of the former three reagents and that of bisulfite probably reflects a difference in the equilibrium constants for the reactions involved. It is known that under some conditions bisulfite produces one sulfhydryl and one S-sulfonate group from each disulfide (8). This possibility, together with the slow rate of the bromate reaction in dough, may explain the observation that the effect of bisulfite on the physical properties of gluten cannot be entirely overcome by bromate (5).

Thiolated gelatin was not included in the comparison made above because it is difficult to ascertain from the present study whether the observed increase in bromate reaction reflects a reaction of bromate ions with the sulfhydryls of thiolated gelatin or with the sulfhydryl produced by reaction of thiolated gelatin with gluten disulfide. It may well be that, so far as the bromate reaction is concerned, the origin of the sulfhydryl groups is immaterial.

Sodium borohydride, which apparently reduces disulfide groups of wheat protein to two sulfhydryls (4), seems to be an order of magnitude less effective (note the change in scale in Fig. 3) than the other reagents in its ability to increase the bromate reaction. A possible explanation of this observation may be the instability of borohydride in acid solution or its reaction with some extraneous substance. If this is true, then the results of the present study indicate that the amounts of the reagent that actually react in dough with disulfide groups are only about 5% of the amounts used (given in Table I and Fig. 3).

The technological implications of the results presented in this paper deserve comment. It has already been reported that doughs having certain desirable mixing characteristics can be produced by using L-cysteine in conjunction with bromate (6). Accordingly, with the increased understanding of the bromate reaction and its role in determining the physical properties of dough, it might be worth while to re-examine the use of combinations of reducing and oxidizing reagents in baking technology.

From the theoretical viewpoint, the results obtained are consistent with the hypothesis that bromate ions react principally with sulfhydryl groups and the number available is increased by the reagents studied. Moreover, the study provides further evidence that both the sulfhydryl and the disulfide groups are implicated in the improver action of bromate in breadmaking.

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THE BROMATE REACTION IN DOUGH

V. Effect of Flour Components and Some Related Compounds[1]

W. Bushuk and I. Hlynka

ABSTRACT

The disappearance of bromate in dough from a high-grade flour occurs by the reaction of sulfhydryl groups of flour proteins with the bromate ion. Starch and pentosans are not directly involved in the bromate reaction. Crude lipids seem to be involved indirectly. In doughs mixed in air, the lipids use up part of the available oxygen in the dough, probably by the lipoxidase-catalyzed peroxidation reaction, and, accordingly, decrease the inhibitory effect of the oxygen on the bromate reaction. Cumene hydroperoxide added to a dough mixed under nitrogen inhibits the bromate reaction; thus, lipid hydroperoxides, if produced in sufficient quantity, probably can compete with bromate for the sulfhydryl group. It is postulated that the involvement of lipids in the improver effect is through the reaction of sulfhydryl with hydroperoxides produced by the lipoxidase-catalyzed oxidation of lipids. n-Propyl gallate and butylated hydroxyanisole, common antioxidants, exert an inhibitory effect on the bromate reaction in doughs mixed in air but are inactive in doughs mixed in nitrogen. A reaction scheme explaining the role of various flour components in the sulfhydryl-bromate reaction in dough is proposed.

Previous studies of the disappearance of bromate in dough have shown that the reaction seems to depend on the protein content of the flour (1,5). There is some evidence that the ash-producing com-

[1] Manuscript received December 27, 1960. Paper No. 199 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Canada. Presented at the 46th annual meeting, Dallas, Texas, April 1961.

ponents (5) and lipids (6,9) might be involved in this reaction, also. It is now established that, in a dough from a high-grade flour, bromate ion reacts with sulfhydryl groups (2,4,7); however, it is not clear whether these groups are entirely located in the flour proteins, as has been presumed, and whether other flour components are involved in the bromate reaction, either directly or indirectly.

The study described in this paper re-examines the effect on the bromate reaction in dough of proteins and lipids and examines the effect of starch, pentosans, cumene hydroperoxide, and two commercial antioxidants, n-propyl gallate and butylated hydroxyanisole.

Materials and Methods

The flour used for most of this study was a hard red spring, straight-grade flour of 13.2% protein and 0.46% ash (14% moisture basis). In one series of experiments on the effect of protein content, low- and high-protein fractions from a hard red winter flour prepared by the air-classification process and supplied by The Pillsbury Company were used. The low and the high fractions had protein contents of 10.8 and 20.3% and ash contents of 0.40 and 0.68% (14% moisture basis). The average size of the particles comprising these fractions was 21.6 and 4.8 microns, respectively.

The flour components used in this study and the methods used for their separation from the hard red spring flour are described in the paragraphs below.

Granular Starch. The flour was mixed into a dough, and the starch was washed out by working the dough by hand in a beaker of tap-water. Excess water was decanted after centrifugation for 30 minutes at 2,000 r.p.m. The squeegee starch was separated from the granular starch by resuspending in water and centrifuging. The granular starch was then air-dried to a moisture of about 12%. This method yielded granular starch having protein and ash contents of 0.2 and 0.15%, respectively.

Gluten. The gluten was washed out by hand, dried by lyophilization to about 9% moisture, and ground on a Wiley mill to pass through screen No. 30. Two types of gluten were used in this study. One type, henceforth referred to as nitrogen-washed gluten, was prepared in the total absence of oxygen. The dough was mixed under nitrogen and subsequently worked up in a beaker of distilled water, which was initially purged of oxygen by bubbling nitrogen through it. The bubbling of nitrogen was continued throughout the washing. The protein content of the nitrogen-washed gluten was 87% (dry basis).

The second type of gluten, which will be referred to as air-washed gluten, was prepared from a dough mixed in air. The gluten was washed out in distilled water containing normal amount of atmospheric oxygen. The protein content of the air-washed gluten was 88% (dry basis).

Pentosans. The pentosans were prepared by the method of Pence, Elder, and Mecham (10). Nine grams of pentosan material were obtained from 1 kg. of flour.

Lipids. Crude fat was extracted from the flour with petroleum ether (Skellysolve F 95) in a large Soxhlet extractor. The solvent was initially purged of oxygen by bubbling nitrogen through it. Oxygen was excluded from the apparatus during extraction by a continuous flow of nitrogen directed into the top end of the condenser. The extracted fat was recovered by evaporation of the petroleum ether *in vacuo*, and the residual flour was dried in a stream of nitrogen. The fat extracted by this method was about 1% of original flour.

Other materials used in this study were cumene hydroperoxide (Monomer-Polymer, Inc., Leominster, Mass.), n-propyl gallate (practical, Eastman), and butylated hydroxyanisole (Nutritional Biochemicals).

The effects of the flour components, with the exception of lipids, cumene hydroperoxide, and the two antioxidants, were studied by determining the rate of bromate disappearance in doughs prepared from flours obtained by blending together the required amount of the dry component in question and the parent flour. With the high- and low-protein fractions of the hard red winter flour, the protein range was extended by adding granular starch to the low- and the high-protein flours. Two flours, with protein contents between the low- and the high-protein fractions, were prepared by blending required amounts of the low- and high-protein flours. The lipids, cumene hydroperoxide, n-propyl gallate, and butylated hydroxyanisole were added directly to the flour at the time of the preparation of the dough.

Protein contents (N × 5.7) of the blended flours were determined by the Kjeldahl method and will be given in the next section on a dry matter basis.

The doughs in this investigation were all mixed to an absorption of 60% and in an atmosphere of nitrogen except for a small number of doughs used to study the effect of lipids and antioxidants, which were mixed in air. All experiments were made at 30°C.

The initial potassium bromate concentration used was 18.75 mg. per kg. of dough (30 p.p.m. of flour), and the residual bromate was

determined by the method described previously (1). The first-order specific rate constant was used as a measure of the reaction rate (3).

Results of this investigation will be presented in two main sections. First subsection under "Results and Discussion" will describe and briefly discuss the results obtained with flours of different protein content, regardless of whether they were prepared by adding gluten to the parent flour or by diluting the parent flour with starch or pentosan material, or by blending high- and low-protein flours. The second subsection will deal with the results of the experiments on the effect of lipids and the related substances. A second main section will be a general discussion of the interrelationship of the results of the entire investigation.

Results and Discussion

Effect of Protein Content. Table I gives the results for the reaction of bromate ion in doughs from hard red spring flours of different protein content. The components and the protein contents of the blended flours are given in the first and second columns, and the third column gives the specific rate constants. Figure 1 shows graphically the relation between the rate constant and the protein content. The points for the flours enriched with nitrogen-washed gluten fall on the same straight line that passes through the points for the flours prepared by dilution with starch, as well as the point at the origin which represents the bromate reaction in a concentrated starch-water mixture. The two points for the flours enriched with air-washed gluten, together with the point for the parent flour, also give a straight line. However, the increase in the bromate reaction produced by the air-washed gluten is only about 50% of the increase produced by the nitrogen-washed gluten.

These results suggest that about 50% of the sulfhydryl groups of

TABLE I

SPECIFIC RATE CONSTANTS FOR HARD RED SPRING FLOURS OF DIFFERENT PROTEIN CONTENTS

FLOUR OR BLEND	PROTEIN CONTENT	$k \times 10^5$
	%, dry basis	*sec^{-1}*
Flour + N_2 gluten	36.2	5.66
Flour + N_2 gluten	28.1	4.30
Flour + air gluten	39.5	4.10
Flour + air gluten	30.2	3.49
Flour	15.3	2.82
Flour + starch	11.1	1.82
Flour + starch	5.27	0.76
Starch	0.24	0.0

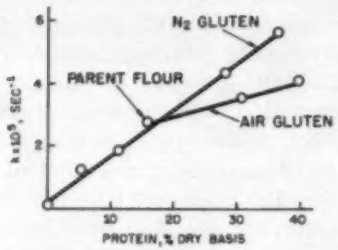

Fig. 1. Rate constants for bromate reaction in doughs from hard red spring flours of various protein content obtained by dilution with starch or by fortification with nitrogen-washed or air-washed gluten.

gluten are lost during the preparation of the dough and the removal of the gluten by washing, if these operations are made under normal atmospheric conditions. This estimate of the sulfhydryl loss agrees with the 50% loss obtained by Sokol, Mecham, and Pence (11) from direct measurements of sulfhydryl content in doughs subjected to prolonged mixing in air. Nitrogen-washed gluten, on the other hand, appears to have retained all of the original sulfhydryl content. These results suggest that it is necessary to use anaerobic conditions to prepare gluten that will retain its natural properties.

Table II gives the results of the experiments with the low- and the high-protein fractions obtained from the same parent hard red winter flour by the air-classification process, two flours obtained by blending the low- and high-protein fractions, and the flours obtained by diluting the low- and high-protein fractions with starch.

Figure 2 shows the variation of the specific rate constant as a function of the protein content for the flours listed in Table II. Linear relationships again result for each set of flours prepared by blending

TABLE II

SPECIFIC RATE CONSTANTS FOR THE LOW- AND HIGH-PROTEIN HARD RED WINTER FLOURS AND BLENDS OBTAINED FROM THEM

FLOUR OR BLEND	PROTEIN CONTENT	$k \times 10^5$
	%, dry basis	*sec⁻¹*
High-protein	23.8	3.81
High-protein + low-protein	20.2	2.78
High-protein + low-protein	16.9	2.07
High-protein + starch	15.8	2.31
High-protein + starch	7.9	1.04
Low-protein	12.4	0.91
Low-protein + starch	9.7	0.74
Low-protein + starch	5.4	0.39

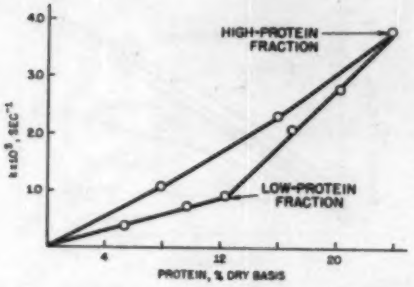

Fig. 2. Rate constants for bromate reactions in doughs from hard red winter flours of various protein content. Upper curve, high-protein fraction diluted with starch; left portion of lower curve, low-protein fraction diluted with starch; right portion of lower curve, blends of high- and low-protein fractions.

any two components. An interesting feature of the results of Fig. 2 is the difference in the slopes of the lines for the flours obtained by diluting the low-protein fraction with starch and those obtained by blending various amounts of the low- and high-protein fractions. Apparently the specific reactivity (the number of sulfhydryls available or accessible for reaction) of the protein in the high-protein fraction is higher than that of the low-protein fraction. This higher reactivity might be a direct result of a shift of the protein of higher sulfhydryl content to the high-protein fraction during the air-classification process, or it might be due to the higher specific surface of the same fraction. It was shown previously that physical accessibility of sulfhydryls, i.e., the specific surface area, plays a definite role in controlling the rate of the bromate reaction in a resting dough (3).

Pentosans do not seem to play any direct role in the bromate reaction other than as a relatively inert diluent similar to starch. Additions of 0.5 and 2% (flour basis) of the pentosan material, to give flours of approximately two and four times the natural pentosan content, did not decrease the protein concentration sufficiently to affect the rate of bromate reaction. It is of interest to note that the added pentosan material produced a definite change in the handling properties of the dough. Doughs from pentosan-rich flours were more sticky than normal doughs.

Effects of Lipids and Related Substances. Figure 3 compares the bromate reactions in the following doughs from hard red spring flour: control doughs from the original flour mixed in nitrogen and in air; doughs from defatted flour mixed in nitrogen and in air; and a dough mixed in nitrogen from defatted flour after reconstitution of the recovered crude fat. The main role of the crude fat (Fig. 3) seems to be

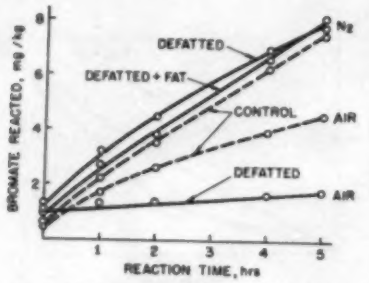

Fig. 3. Effect of crude lipids in the presence and absence of oxygen on the bromate reaction in doughs from hard red spring flour.

related primarily to the effect of oxygen. When the doughs are mixed in nitrogen (top three curves), fat has little effect. In the presence of oxygen (lower two curves), the depression of bromate reaction is much greater in the dough from defatted flour than in the dough from the control flour. The crude fat, probably through the lipoxidase-catalyzed reaction (13), seems to compete with protein sulfhydryl for the available oxygen in the dough, and, hence, the oxygen inhibition of the bromate reaction in the presence of lipids is not as great as in their absence.

Previous studies (6,9) showed that the removal of crude fat in the presence of atmospheric oxygen produced a slight depression in the bromate reaction in a dough from the defatted flour. When the fat is extracted with petroleum ether in an atmosphere of nitrogen the bromate reaction is not affected; actually, a slight increase was observed in the present study. This is ascribed to removal from the flour of certain inhibitory substances, e.g., hydroperoxides (see below).

Attempts to produce a totally defatted flour by extraction with water-saturated n-butanol were unsuccessful because it was not possible to remove the last traces of butanol from the residual flour without damaging its dough-forming properties.

To determine the possible role of lipid hydroperoxides in the bromate reaction, experiments were made with doughs containing up to 2.5 mg. per g. of flour of cumene hydroperoxide, which might be expected to compete with bromate for the available sulfhydryl groups. As was expected, a definite competitive inhibition of the bromate reaction was obtained. Accordingly it is concluded that lipid hydroperoxides, if produced in sufficient quantity, would react with protein sulfhydryl and in this way bring about an improver effect.

The role of antioxidants in the bromate reaction also seems to involve atmospheric oxygen. Figure 4 suggests that n-propyl gallate has

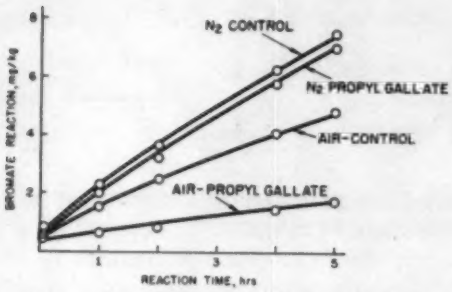

Fig. 4. Effect of n-propyl gallate in the presence and absence of oxygen on the bromate reaction in doughs from hard red spring flour.

essentially no effect in a dough mixed in nitrogen, whereas in a dough mixed in air it acts as an inhibitor. Similar results were obtained with butylated hydroxyanisole. Accordingly, any antioxidants occurring naturally in flour might be expected to behave similarly.

General Discussion

Results of this investigation are in agreement with the hypothesis that the sulfhydryl groups which react with the bromate ion in doughs from a high-grade flour are an integral part of the flour proteins. Starch and pentosans are not involved in the bromate reaction except as inert diluents of the protein. Lipids play a secondary role by reacting with part of the available oxygen and thus lessening the inhibitory effect of oxygen on the bromate reaction. By analogy with the effect of cumene hydroperoxide, which apparently competes with bromate for the available sulfhydryls, the hydroperoxides produced by the lipoxidase-catalyzed oxidation of fats might also be expected to react with sulfhydryl groups in dough. Antioxidants, in the presence of oxygen, depress the bromate reaction; accordingly it is the oxidation product that reacts with the sulfhydryl, as was the case with lipids.

Although the kinetics of the bromate reaction in dough seem to be complex, the results that have been obtained in the present investigation of this reaction can be summarized schematically by the following sets of competing reactions:

Reactions competing for sulfhydryl:

$$\text{Protein -SH} + \begin{cases} BrO_3^- & (1) \\ O_2 & (2) \\ \text{hydroperoxides} & (3) \\ \text{oxidized antioxidants} & (4) \end{cases} \longrightarrow \text{Products}$$

Reactions competing for oxygen:

$$\text{Oxygen} + \left\{\begin{array}{l}\text{Protein -SH} \\ \text{Lipids} \\ \text{Antioxidants}\end{array}\right\} \longrightarrow \text{Products} \qquad \begin{array}{l}(2) \\ (5) \\ (6)\end{array}$$

In a bromated dough mixed in nitrogen, the main reaction is undoubtedly that given by equation 1. However, in the more normal situation where the dough is mixed in air, with incorporation of a certain amount of oxygen, all six reactions probably occur. The most important reactions seem to be 1, 2, and 5, although the others may well occur to a limited extent.

The involvement of the sulfhydryl group of the cysteine residues of flour proteins in the chemical improvement of flour quality seems to be well established. Whether this improver effect is produced by the cross-linking mechanism first proposed by Sullivan, Howe, Schmalz, and Astleford (12), the blocking mechanism of Goldstein (8), or some other mechanism is still a matter of conjecture. Final decision as to which mechanism predominates for a particular improver must await further basic study of this problem.

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ELECTROPHORETIC COMPOSITION AND INTRINSIC VISCOSITY OF GLUTENS FROM DIFFERENT VARIETIES OF WHEAT[1]

J. E. Cluskey, N. W. Taylor, Helen Charley,[2] and F. R. Senti

ABSTRACT

The protein compositions of hard and soft wheat flours were compared by electrophoresis of the gluten and water-soluble constituents fractionated from selected samples of the defatted flours. Although the amount of total protein was higher in the hard wheat than in the soft wheat flours, the amount of water-solubles, as recovered from gluten washing, was essentially the same. Hard wheat flours contained more of the electrophoretic components alpha, gamma, and omega but the same amounts of beta and fast components. A comparison based on gluten rather than on the flour was also made. When defined as the sum of the alpha, beta, gamma, and omega components, the gluten proteins of the hard wheat flours contained more alpha, less beta, and identical amounts of gamma and omega components. The reported differences were small but statistically significant. Purified gluten fractions from the hard wheats exhibited the greater intrinsic viscosities. This difference probably reflects the higher alpha content in the hard wheat class.

Variations in composition of proteins from different varieties of wheat have been investigated by many cereal chemists (3). Chemical analyses and physical-chemical methods have been employed. One of the more important physical techniques for the characterization of protein mixtures is moving-boundary electrophoresis. Wheat gliadin electrophoresis studies were reported by Schwert, Putnam, and Briggs (6) in 1944. A review of wheat protein electrophoresis has recently been made by Abbott (1).

In 1948 Laws and France (5) described electrophoresis studies on four hard red winter wheat glutens. This comparative study showed no significant difference in the gluten protein of several good and poor quality flours. Unlike their investigation, the aim of this work was to find whether significant electrophoretic compositional differ-

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ences exist between hard and soft wheat gluten proteins.

The lack of good buffer systems for gluten electrophoresis has been a major handicap in using this analytical tool for such investigations. Recently, Jones, Taylor, and Senti (4) described a buffer system that produced symmetrical patterns in the ascending and descending limbs. This advance now offers a better opportunity for detecting possible differences in the composition of wheat proteins.

Materials and Methods

The basic materials used in this study were nine samples of hard red winter wheat flour representing three different varieties grown at three locations, and eight varieties of soft wheat flour. The hard wheat varieties were Concho, Ponca, and CI 12871; they were grown at Manhattan, Powhattan, and Hutchinson, Kansas. The soft wheat class was represented by American Banner, Thorne, Clarkan, Trumbull, Kawvale, Fairfield, Blackhawk, and Wabash, all grown at Wooster, Ohio. All flours were Buhler-milled, straight grade flours and were from the 1957 crop. The protein range of hard wheat flours was 10.8 through 14.9% and that of the soft flours, 9.0 through 10.5%. Duplicate fractionations and analyses were made on some of the flour samples.

The method of separating the gluten and water-soluble fractions from the wheat flours differed in minor respects from that recently described by Jones and co-workers (4). Starch and other nongluten materials were washed from 25 g. of butanol-defatted flour by kneading the dough ball between two stirring rods in multiple 10-ml. aliquots of 0.1% sodium chloride solutions. All solutions and containers were chilled in an ice water bath. The wash solution containing the water-soluble materials was decanted into centrifuge tubes and centrifuged (2,000 × G). After the supernatant solutions were collected, the remaining starch cake was slurried with additional sodium chloride solution to assure dissolution of any remaining solubles. After a second centrifugation, the supernatant was combined with the first, and the starch remaining was discarded. The combined water-soluble fraction was lyophilized.

The ball of washed gluten was dispersed at about 4% protein concentration in 0.01*N* acetic acid in a Waring Blendor[3]. The dispersion was centrifuged at 21,000 × G. The clear supernatant solution was heated to 98°–100°C., cooled quickly, and then lyophilized. Protein was determined by the Kjeldahl procedure (N × 5.7) for all the isolated gluten and soluble fractions, as well as for all the defatted

[3] Reference to specific equipment or organizations does not necessarily constitute endorsement by the U. S. Department of Agriculture.

flour samples.

Solutions for electrophoresis were prepared by dissolving known weights of the gluten and water-soluble fractions in 0.017*M* aluminum lactate-lactic acid buffer, pH 3.2, conductivity 600 to 610 micromhos at 1°C. The aluminum lactate was a recrystallized preparation of commercial aluminum lactate. Further recrystallization did not alter the conductivity. Average solution concentrations used were 0.5% for the gluten and 0.4% for the water-solubles. Dialysis in a large volume of buffer stored in a refrigerator preceded electrophoresis. Dilution was determined by weighing the dialysis bags before and after dialysis. The gluten solutions were then filtered through coarse fritted funnels and stored in the cold in tightly stoppered bottles; the water-soluble solutions were not filtered but were centrifuged for 10 minutes in a Sorvall laboratory centrifuge.

Electrophoresis runs were carried out at 1°C. in a Spinco Model H electrophoresis apparatus equipped with a cylindrical-schlieren lens system and a Rayleigh interference system. Gluten solutions were run for 130 minutes and the water-solubles for 90 minutes, both at 8.8 v. per cm. Photographs of the moving boundary were taken at regular intervals. The relative concentration of each component was determined from the relative number of Rayleigh fringes lying below the peak in the schlieren diagram. Fringes in the delta and epsilon boundaries were ignored in this calculation.

Total protein concentration was calculated from the total number of fringes in the gluten fractions and from the fringes corresponding to the moving boundaries in the water-soluble fractions, using a specific refractive increment of 0.00188, in units of 100 ml. per g. With a 2.5-cm. thick cell, 172 fringes corresponded to 1% gluten concentration. The protein in the original weighed sample of fraction was calculated from the concentration in solution after correction for dilution during dialysis.

Viscosity measurements were made only on the gluten fraction using the same solution as for electrophoresis. Flow times of the original solution and of three dilutions were determined in Ostwald viscometers at 24.9°C., constant within 0.01°C. The values of intrinsic viscosity were then derived from a plot of $(\eta_{rel}-1)/c$, with c as the concentration and η_{rel} the ratio of outflow times of solution to solvent. The concentration of the undiluted gluten solution was determined by differential refractometer measurements.

Results

Kjeldahl analyses of the defatted flour samples are summarized in

TABLE 1

Fractionation and Electrophoretic Composition of Flours and Intrinsic Viscosity of Gluten Fractions

Variety	(A) Protein by Kjeldahl in:			Weight Percentage Found in Flour of 14% Moisture										(C) Intrinsic Viscosity
				(B) Electrophoretic Components Found										
	Flour	Gluten	Water Solubles	f_D[a]	f_A	Alpha		Beta		Gamma		Omega		
						D	A	D	A	D	A	D	A	
Hard:														
Concho M[b]	10.84	8.08	1.04	1.28	1.17	4.79	4.34	1.43	1.40	0.87	1.36	0.11	0.23	0.33
Concho H-1[c]	11.52	8.68	1.08	0.98	0.87	4.73	4.31	1.06	1.36	1.06	1.29	0.23	.19	.39
Concho H-2	11.52	8.80	1.04	1.17	1.10	5.45	4.99	1.17	1.51	1.44	1.55	0.15	.23	.35
Concho P	13.54	10.32	1.12	1.32	1.13	5.71	5.18	1.93	2.08	1.02	1.25	0.19	.45	.37
CI-12871-M	11.45	8.40	1.08	1.18	1.06	5.04	4.47	0.72	1.55	1.63	1.36	0.15	.19	.38
CI-12871-H-1	11.95	9.32	1.00	1.13	1.09	5.99	5.16	1.58	1.85	1.24	1.51	0	.30	.34
CI-12871-H-2	12.06	9.44	1.00	1.09	1.02	5.84	5.32	1.17	1.43	1.58	1.96	0.30	.30	.32
CI-12871-P	14.75	11.12	1.16	1.25	1.17	7.05	6.00	1.28	1.96	2.19	2.19	0	.42	.35
Ponca M-1	11.92	8.68	1.12	1.25	1.14	5.18	4.65	1.14	1.48	1.40	1.51	0.19	.23	.31
Ponca M-2	11.37	8.32	1.08	1.29	1.25	5.14	4.58	0.91	1.40	1.51	1.55	0.19	.19	.34
Ponca H-1	11.62	8.60	1.04	1.44	1.21	5.21	4.76	1.32	1.62	1.36	1.55	0.11	.30	.35
Ponca H-2	11.75	8.64	1.08	1.25	1.13	5.29	4.72	1.25	1.59	1.32	1.51	0.26	.34	.33
Ponca P	14.85	11.24	1.28	1.36	1.25	6.98	6.30	1.81	2.00	1.58	2.15	0.38	0.38	0.36
Soft:														
Am. Banner-1	8.87	5.96	1.08	1.19	1.11	3.42	3.11	1.04	1.23	0.88	1.08	0.19	0.19	0.35
Am. Banner-2	9.25	6.52	1.12	1.11	1.04	3.46	3.11	1.04	1.27	0.81	1.08	0.23	.23	.28
Thorne	9.05	6.92	1.08	1.31	0.96	4.16	3.96	1.08	1.35	1.04	1.19	0.23	.23	.30
Clarkan-1	10.01	7.64	1.04	1.23	1.15	4.52	4.02	1.38	1.65	1.07	1.34	0.19	.23	.35
Clarkan-2	9.80	7.88	1.04	1.19	1.11	4.14	3.71	1.34	1.57	1.00	1.23	0.15	.15	.31
Trumbull	9.27	7.24	1.04	0.88	0.88	4.68	4.15	1.11	1.46	1.15	1.34	0.12	.15	.31
Kawvale	10.04	7.48	1.08	1.19	1.15	4.46	3.92	1.12	1.31	1.23	1.38	0	.23	.30
Fairfield	9.33	6.80	1.08	1.20	1.12	3.94	3.59	1.04	1.20	1.00	1.20	0.19	.15	.30
Blackhawk-1	10.46	8.12	1.16	1.34	1.22	4.21	3.79	1.15	1.49	1.26	1.38	0.19	.19	.29
Blackhawk-2	10.48	8.16	1.12	1.42	1.26	4.44	4.05	0.99	1.49	1.61	1.57	0.19	.19	.30
Wabash	9.40	7.04	1.08	1.34	1.15	3.82	3.55	1.15	1.49	0.99	1.03	0.19	0.23	0.28

[a] The letters D and A refer to descending and ascending limbs of the electrophoresis cell.
[b] The letters M, H, and P refer to wheat grown at Manhattan, Hutchinson, or Powhattan, Kansas.
[c] The numbers (1) and (2) refer to analyses on duplicate fractionations of the same flour sample.

Table I(A). Recovery of nitrogen in the gluten and water-soluble fractions is also given. About 10 to 20% of the nitrogen was lost in fractionation. Experiments on a Ponca flour indicated that about one-third of this unaccounted-for protein was in the discarded starch, and about two-thirds was acid-insoluble material which did not go into solution in acetic acid.

Figure 1 shows typical electrophoresis patterns of a hard and soft wheat gluten and the corresponding water-soluble fraction. Only ascending limbs are shown because in the aluminum lactate buffer the limbs are nearly identical.

Five peaks, designated alpha, beta, gamma, omega, and delta, appear in the gluten electrophoresis patterns. Other workers have reported that two alpha peaks (4) were resolved in moving-boundary electrophoresis of Ponca gluten in other buffer systems. Also, four beta peaks (8) have been resolved in the starch gel electrophoresis of Ponca gluten. However, in the moving-boundary system using aluminum lactate buffer, the alpha components yield only one peak. In one case, mentioned below, two beta peaks were observed. The components are treated as single species units in this work. The delta peak represents the salt boundary. The material preceding the main gluten

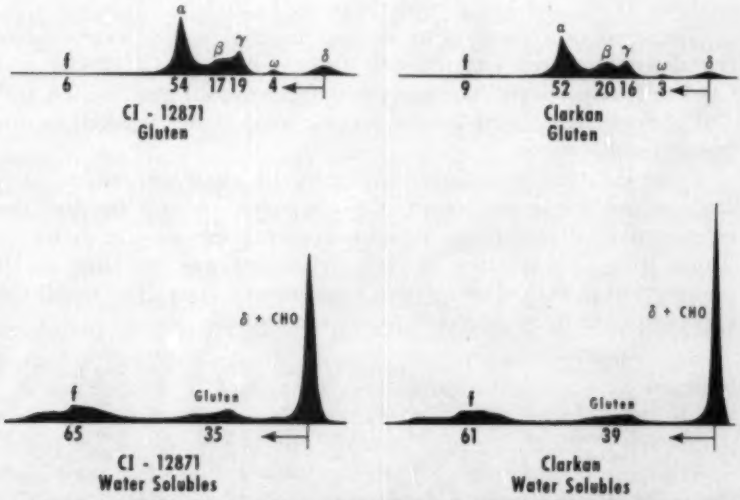

Fig. 1. Ascending limb schlieren diagrams with component concentrations for hard wheat (CI-12871-P) gluten and water-solubles and for soft wheat (Clarkan-1) gluten and water-solubles. Components are designated as α (alpha), β (beta), γ (gamma), ω (omega), and f (fast). The position of the initial boundary and immobile material is δ (delta).

protein group is designated as *f*, indicating fast moving. The value given under each peak (Fig. 1) is the percentage of component calculated from the ascending pattern.

The pattern of the water-solubles shows three main regions. The fastest moving component labeled *f* is evidently heterogeneous material, which probably contains the albumin and globulin components. The middle section is the gluten area, so labeled because of similar mobilities to isolated gluten. The large stationary peak is a composite of the salt boundary and soluble carbohydrate material. This large peak is not included in the calculation of protein concentration.

Relative concentrations of components measured somewhat differently in the two limbs of the electrophoresis cell. In Table I(B) are given the total amounts of each component in the flours. These figures are the sums of the protein contained in the gluten and water-soluble fractions. The totals calculated in ascending and descending limbs are given separately.

In the typical electrophoresis analysis given in Fig. 1, the proportionation among the components in each fraction is shown. The total concentration of protein found in the gluten solution corresponded to 10.9% of the weight of 14%-moisture flour for CI 12871-P, and 7.6% of the flour for Clarkan-1. These values agree with the Kjeldahl analyses, 11.1% and 7.6%, respectively, in Table I(A).

The total concentration of moving material in the water-soluble fractions represented 0.83 and 0.80% of the flour for CI 12871-P and Clarkan-1, respectively. The corresponding Kjeldahl analyses, 1.6 and 1.04%, respectively, were greater because some of the nitrogen in this fraction is dialyzable.

From the total protein accounted for by electrophoresis of each fraction and the proportion of each component in each fraction, the calculated total percentage amount of each component is shown in Table I(B). The mobility of each component was the same in all samples and in both classes within experimental error. The mobilities expressed in units of 10^{-5} cm.^{2} per volt-second are:

Component	*Ascending Limb*	*Descending Limb*
Alpha	5.58	5.26
Beta	3.95	3.79
Gamma	3.26	3.07
Omega	1.94	1.40

These values are somewhat lower than those of Jones *et al.* (4). The difference lies in the concentration of aluminum lactate, which was somewhat lower in their work, as shown by the conductivity of the buffers.

Fig. 2. Ascending limb schlieren diagram of hard wheat (Concho P) gluten showing a double beta peak (β_1 and β_2).

Figure 2 shows the ascending limb for electrophoresis of hard wheat Concho P gluten. This gluten clearly demonstrates a double beta peak. Although splitting of the beta peak was not unusual after 2 to 3 hours' electrophoresis, this is the only gluten from the 17 surveyed that showed the double beta peak throughout the run. The Concho P variety had the highest content of beta in flour and in gluten.

Discussion

The results reported in Table I show that there were no gross qualitative differences among the various flours investigated, either in kind of electrophoretic components present or even in relative amounts of components. Analysis of variance was applied to determine significant sources of variation in the data.

These analyses are subject to the usual uncertainties of electrophoresis. The different peaks were only partially resolved, and the results in the two different limbs of the electrophoresis cell were not identical. Further, the concentrations as measured across the boundaries of any individual component may be considerably biased compared to the true concentrations of component (2). In this study, however, the various samples were fractionated and analyzed under identical conditions, and any bias not taken into account by the statistical procedure should affect all analyses to about the same extent.

Fractionation. The mean values of nitrogen analyses of the fractions are summarized for each class in Table II. The probabilities, P, are shown for significance of the variance between classes, compared to that of samples within a class, and also for significance of the variance between samples in a class, compared to that between duplicate fractionations.

The hard wheat flours contained the higher percentage total protein and gluten, showing that the samples were normal in terms of

TABLE II

PERCENTAGE DISTRIBUTION OF NITROGEN (AS PROTEIN) IN FRACTIONS ISOLATED FROM HARD AND SOFT WHEAT FLOURS (14% MOISTURE) AND ANALYSIS OF VARIANCE

MATERIAL	MEANS, % OF FLOUR		P, %	
	Hard	Soft	Between Classes vs. Samples in Class	Between Samples in Class vs. Duplicates
Total protein	12.24	9.63	>99.95	>99.95
Gluten	9.20	7.25	>99.95	>99.95
Water-soluble	1.09	1.08	NS[a]	98
Unaccounted	1.95	1.30	>99.95	97.5

[a] NS denotes a probability (P) value of less than 90%.

protein content.

The water-soluble fraction was less variable among samples, however, and no significant difference was found between these fractions in the two classes of flour. The relation of the *f* material in this fraction to the gluten components is discussed under electrophoretic composition.

Finally, the amount of unaccounted nitrogen was greater in the hard wheat flours than in the soft. This result may indicate a difference in protein composition since the unaccounted nitrogen is also a larger fraction of the total nitrogen in hard wheats. There is some evidence in other work that this material is a different protein or, perhaps, an insoluble alpha component since, in one case examined in detail, the unaccounted material was largely acid-insoluble.

Electrophoretic Composition. The mean amounts of each component found in the flours are summarized for the two classes in Table III. The P values, for comparison of variances between classes with those of samples in a class, were high for all components except *f*, which appears to be relatively nonvariant. This material represents two-thirds of the water-soluble fraction (Fig. 1), which was relatively nonvariant compared to the other fractions. The different amino acid composition of the water-soluble fraction, as determined by Woychik

TABLE III

MEAN PERCENTAGES OF ELECTROPHORETIC COMPONENTS IN FLOURS (14% MOISTURE) AND ANALYSIS OF VARIANCE

COMPONENT	MEANS, % OF FLOUR		P, %	
	Hard	Soft	Between Classes vs. Samples in Class	Between Samples in Class vs. Duplicates
f	1.18	1.16	NS[a]	NS
Alpha	5.27	3.92	99.95	98
Beta	1.46	1.27	90	93
Gamma	1.50	1.18	98	95
Omega	0.23	0.18	95	NS

[a] NS denotes a probability (P) value of less than 90%.

TABLE IV

MEAN PERCENTAGE OF ELECTROPHORESIS COMPONENTS IN GLUTEN PROTEIN ($\alpha + \beta + \gamma + \omega$)

COMPONENT	MEANS, % OF GLUTEN		P, %	
	Wheat Flours		Between Classes vs. Samples in Class	Between Samples in Class vs. Duplicates
	Hard	Soft		
Alpha	62.34	59.77	>99.95	>99.95
Beta	17.26	19.47	97.5	NS
Gamma	17.68	17.81	NS[a]	NS
Omega	2.72	2.85	NS	NS

[a] NS denotes a probability (P) value of less than 90%.

et al. (7), is additional evidence that the *f* component is distinct from the gluten components.

There were more of the remaining components, alpha, beta, gamma, and omega, in the hard wheat flours. This consequence is largely caused by the greater protein content of hard wheat flours since these components represent most of the protein.

Calculation of component concentration on the basis of gluten (Table IV) provides a better comparison than one on the basis of flour because the direct effect of variation in total gluten protein can thereby be eliminated. The gluten proteins were assumed to be the sums of alpha, beta, gamma, and omega components. Apparently, there is significantly more alpha in hard wheat gluten proteins and less beta. Table IV shows clearly that the gluten proteins do not vary proportionately with the total gluten protein.

The P values, for comparison of variance between samples within the classes with variance between duplicates, are also given in Table IV. These figures show that alpha, especially, was more variable than was ascribable to duplication of fractionated analyses. Evidently glutens are quite definitely variable in composition within a class, too. The analytical method is precise enough to demonstrate this variability between samples even though the variability of gluten composition is relatively small.

Although proteins in the flour samples investigated are significantly variable in composition, there is still some uncertainty in ascribing the source of observed variability to the two classes of wheat. Variability of gluten composition can be the composite of several sources which cannot be differentiated by the statistical procedure. In principle, sources of variance among the flour samples are those between classes, between varieties within a class, and between conditions under which the wheat was grown and handled.

Because samples from the two classes of wheat were grown at two

locations, the variance observed between classes is a composite of the three sources. The variance between the soft wheat samples, all grown at one station, is a composite of two sources: varieties and growing conditions. The samples of hard wheats, three varieties each grown at three locations, can ideally be used to distinguish variance between varieties from variance between growing conditions. When this test was made, neither source was significant for any component of gluten. Therefore, because the variance between the hard wheat samples due to location was not significant and because the variance between classes was relatively large for the alpha content of gluten, it can be tentatively concluded that the two classes of wheat have glutens of different composition.

The soft wheat flours contained distinctly less protein than did the hard wheat flours. Because of this difference, it might be postulated that the greater alpha content of the glutens of the hard wheat flour was a function of protein content rather than class of wheat. Information on this point was obtained by determining the coefficient of correlation for α content in gluten with protein content in the flour. This correlation was not significant (P was about 80%), nor were any of the other gluten components correlated with protein content of flour. Thus, based on this tentative evidence, the class of wheat is a more significant source of variation in gluten composition than is protein content of flour.

Another point is the estimation of the possible range in alpha content of glutens of various flours. The observed range was 5% of alpha. Most of this range is attributable to differences among wheat samples because 5% was significantly greater than the variation between duplicates. Furthermore, if the unaccounted material lost in fractionation was also partly alpha, as suggested, then the range of gluten protein composition may be greater than it appears from Table IV since a greater proportion of protein was lost from the hard wheat samples. In any case, enough variation in the alpha component has been found to suggest that some of the variation in gluten properties may result from variability in gluten protein composition. This idea is strengthened by intrinsic viscosity measurements.

Intrinsic Viscosities. In other works to be published[4], the alpha component had a higher intrinsic viscosity than the other major components. The intrinsic viscosity of gluten in aluminum lactate is thus largely dependent on the alpha protein.

Average intrinsic viscosity of the hard wheat glutens was 13% higher than that of the soft wheats, and the two classes were signifi-

[4] Taylor, N. W., Cluskey, J. E., and Senti, F. R., unpublished data.

TABLE V

INTRINSIC VISCOSITIES OF GLUTENS AND ANALYSIS OF VARIANCE

MEANS, 100 ML/G		P, %	
Wheat Flours		Between Classes vs. Samples in Class	Between Samples in Class vs. Duplicates
Hard	Soft		
0.35	0.31	99.8	NS[a]

[a] NS denotes a probability (P) value of less than 90%.

cantly different (Table V). Thus, the higher intrinsic viscosities of the hard wheat flours correlate with their higher alpha content. The glutens of the two classes are definitely different in both protein composition and properties, although this difference is a small one.

Although intrinsic viscosities were 13% greater in the hard wheat glutens, the average alpha content was greater by only 7% of the mean. This fact suggests that the relatively minor variation in components may cause marked changes in physical properties. At the same time, it is not suggested that the main source of variability in gluten properties is protein composition; instead, protein composition is one possible, and rather likely, source of variability.

Acknowledgments

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A LIPOPROTEIN MODEL OF WHEAT GLUTEN STRUCTURE[1]

J. C. GROSSKREUTZ[2]

ABSTRACT

Electron micrographs and X-ray studies of gluten have shown the proteins to exist in platelet form of the order of 70A thick. Extraction of the phospholipids does not affect the basic platelet, but does seriously impair their ability to bond into sheets capable of sustaining large plastic deformations. X-ray evidence of the phospholipid structure in gluten favors the assumption that there exist well-oriented bimolecular leaflets of the type found in myelin figures. On the basis of the information at hand, a lipoprotein model is postulated which occupies some 2 to 5% of the elastic gluten structure. Consideration of the nature and strength of the bonds present in a hypothetical gluten sheet shows that the proposed lipoprotein model is capable of providing gluten with the plasticity necessary for optimum baking characteristics.

The structure responsible for the unique plastoelastic properties of wheat gluten has been investigated, using both X-ray and electron-microscopic techniques capable of structural detection on the macromolecular level. The details of the techniques used have been described in an earlier publication (3) and will not be repeated here. Gluten samples were prepared from two unbleached, untreated flours obtained as pure strains from Kansas State University. One strain (Pawnee) was characterized by a short dough-development time; the other (an experimental strain) by a long development time. Since no significant differences in the physical microstructure between the two glutens from these flours were found (3), no distinction will be made between samples in this paper.

X-Ray and Electron-Microscopic Evidence Concerning Gluten Structure

Previous work (3) carried out at this laboratory has demonstrated that the proteins in gluten consist of folded polypeptide chains in the α_1-helix configuration arranged into flat platelets of the order of 70A thick. Figure 1 shows the typical X-ray scattering at small angles from moist gluten and its excellent agreement with the theoretically predicted platelet scattering. Two diffraction peaks which are visible in the moist gluten curve are interferences produced by the phospholipid component. Their significance will be discussed in detail later in this paper. Also shown in Fig. 1 is the scattering obtained from lyophilized,

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[2] Midwest Research Institute, Kansas City, Mo.

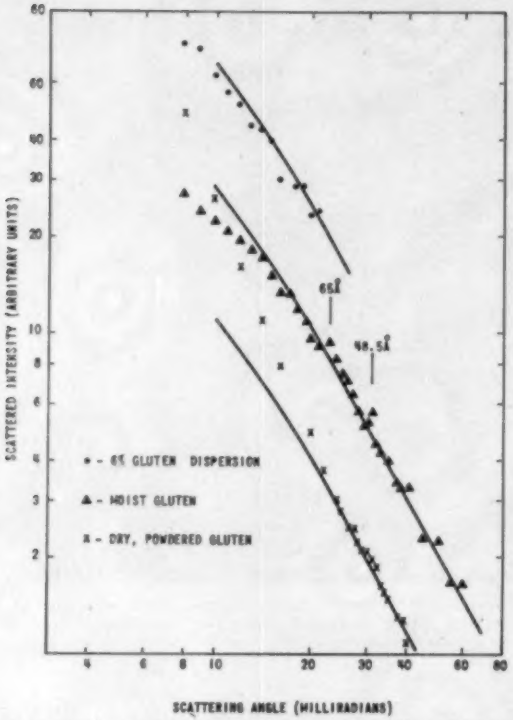

Fig. 1. X-ray scattering at small angles from moist and powdered glutens. Solid curves are the theoretical scattering from platelets.

powdered gluten which shows serious departure from the theoretical platelet scattering, especially at very small angles. This behavior indicates that the moist gluten platelets have, on drying, agglomerated

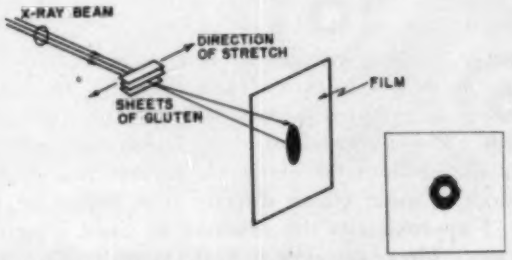

Fig. 2. X-ray scattering at small angles from stretched, freeze-dried gluten showing oriented scattering pattern.

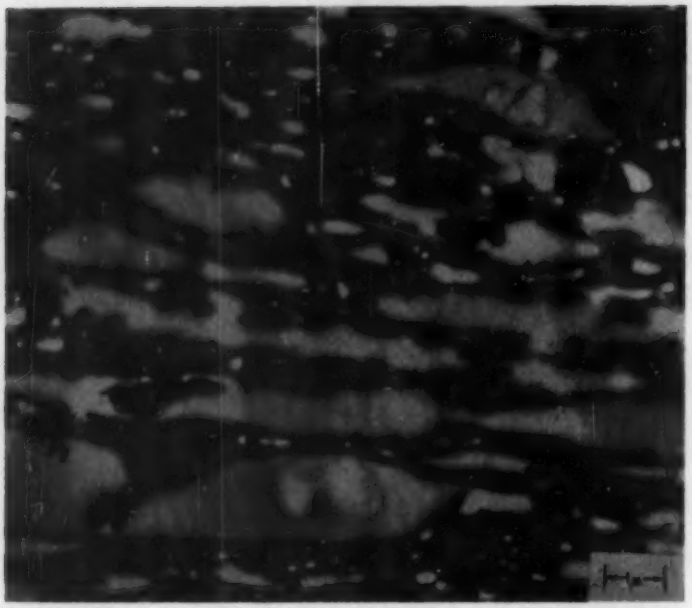

Fig. 3. Cross-section of stretched, dried gluten fixed with (OsO_4). Thin section cut perpendicular to plane of stretch and along direction of stretch. Direction of stretch horizontal, magnification as shown.

into thicker lamellae.

To better understand this structure, consider the scattering obtained from stretched, freeze-dried gluten (3). Figure 2 depicts the oriented scattering pattern as recorded on photographic film. The angular dependence of this scattering, however, is the same as the powdered gluten curves given in Fig. 1. Thus, one may conclude that the units giving rise to the scattering in the powdered sample have, when stretched, arranged themselves into sheets parallel to the plane of stretch. Confirmation of these X-ray findings is afforded by electron micrographs of freeze-dried, gluten thin sections which were cut perpendicular to the plane of stretch and along the direction of stretch. Figure 3 shows the oriented sheetlike structure of a stretched, freeze-dried sample. The thickness of these sheets ranges from a few hundred to several thousand angstroms. Because it is not possible to obtain sections of moist gluten directly, it is impossible to say how closely Fig. 3 approximates the structure of moist gluten. However, since the X-ray evidence certainly indicates considerable agglomeration of the basic protein platelets on drying, one may assume that the

Fig. 4a. Surface replica of stretched, freeze-dried gluten. Direction of stretch horizontal.

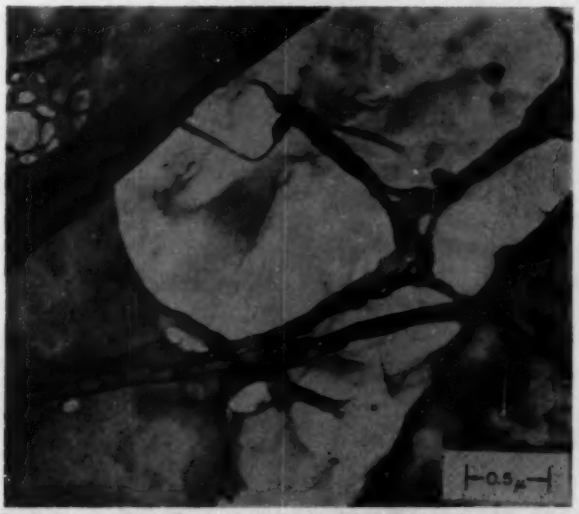

Fig. 4b. Surface replica of stretched, freeze-dried gluten showing three layers of gluten sheet.

picture in Fig. 3 is a distorted one.

The behavior of gluten sheets under strain has been observed by

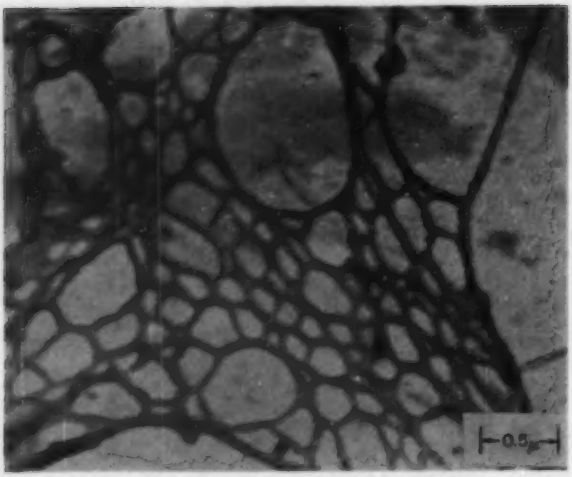

Fig. 4c. Surface replica of stretched, freeze-dried gluten showing extreme example of ruptured sheet.

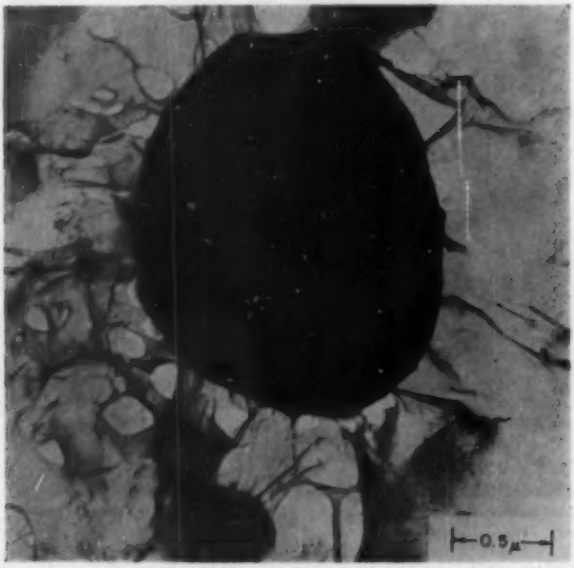

Fig. 5. Surface replica of stretched, freeze-dried dough showing starch grain (large black mass) occluded in matrix of gluten sheet.

means of electron micrographs taken of surface replicas of freeze-dried stretched gluten. Figures 4a, b, and c show different stages in the rupture of a strained sheet into filaments. These tiny filaments measure approximately 200–300 A in diameter.

Of great practical interest is the actual structure of a dough on the macromolecular level. Figure 5 is an electron micrograph of a surface replica of stretched, freeze-dried dough which was mixed to peak consistency. A starch grain is shown held in place by a gluten sheet which has been strained to rupture in certain areas.

The Place of Lipids in Gluten Structure

Background. The effect of lipids, especially phospholipids, on gluten behavior has been a subject of interest and investigation for many years. Working (12,13) found that the presence of phosphatides caused gluten to be soft and plastic. Prolonged washing in water removed a portion of the phospholipids and caused the gluten to stiffen. Adding back wheat phosphatides caused glutens to become softer and more pliable. He concluded that the phosphatides formed a film over the gluten mesh and lubricated it, causing a loss of cohesiveness. Furthermore, he showed that oxidizing agents "liberated" phosphatides and improved their lubricating action. Geddes and Larmour (2) found a close relationship between phosphatide content and the baking behavior of a flour-germ mixture. The statement is made that there appears to be an optimum amount or concentration of phospholipid. An excess results in a soft gluten, a deficiency in a harsh, tough, inelastic gluten. Recently Mecham and Mohammad (6) have shown that lipid extraction with water-saturated n-butanol yields a flour which requires a fourfold increase in development time. Experiments in this laboratory have confirmed the latter observation; it was necessary to enclose the doughball in a nylon bag during washing in distilled water to retain a gluten mass. The coherency of this gluten under strain is very small.

Obviously, then, the presence or nonpresence of lipids, especially phospholipids, has a marked effect on the "quality" of the gluten structure in dough. To understand this effect, it is necessary to inquire more deeply into the structure of phospholipids. Stoeckenius (9) has shown that the pure phospholipids, lecithin and cephalin, extracted from human brain tissue form highly ordered molecular aggregates in aqueous media. Figure 6 illustrates the model proposed by Stoeckenius to account for his electron-microscope observations and the X-ray observations of others. This bimolecular leaflet consists of two phospholipid molecules with their hydrophobic ends inwardly oriented

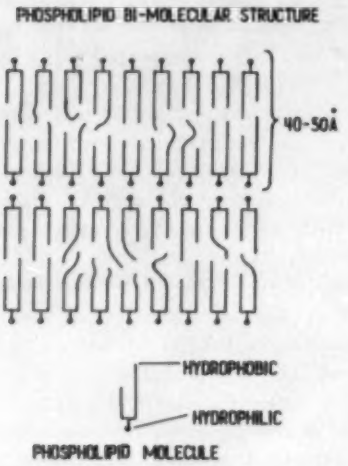

Fig. 6. Phospholipid bimolecular leaflet structure.

and their hydrophilic, phosphoric acid groups outwardly oriented into the aqueous media. The most commonly observed width of the leaflet is 42A.

X-ray Evidence of Lipid Structure in Gluten. The X-ray scattering curve from moist gluten shown in Fig. 1 possesses diffraction maxima characteristic of the phospholipid bimolecular leaf dimensions. It has been shown (3,4,11) that extraction of phospholipids reduces the intensity of these diffraction lines in gluten, and further that the extracted lipids show the same diffraction maxima. The phospholipids must, moreover, be wetted in order to develop the bimolecular leaflet structure. Figure 7 shows the X-ray diffraction pattern of the lipids extracted from dry flour (where presumably no water was available to the lipids) and from developed gluten (in which hydration has occurred thoroughly during mixing).

The author, therefore, assumes that a bimolecular phospholipid leaflet structure exists in dough or glutens. Since the characteristic diffraction lines persist even in dried glutens (3), the structure is fairly well stabilized in the developed gluten. This is completely in agreement with the observations of Olcott and Mecham (7), who demonstrated the difficulty of lipid extraction, especially phospholipids, after doughing.

Effect of Lipid Removal on Platelet and Sheet Structure of Gluten. A simple manual inspection of the gluten extracted from doughs made from flour defatted with n-butanol reveals that the gluten is extremely

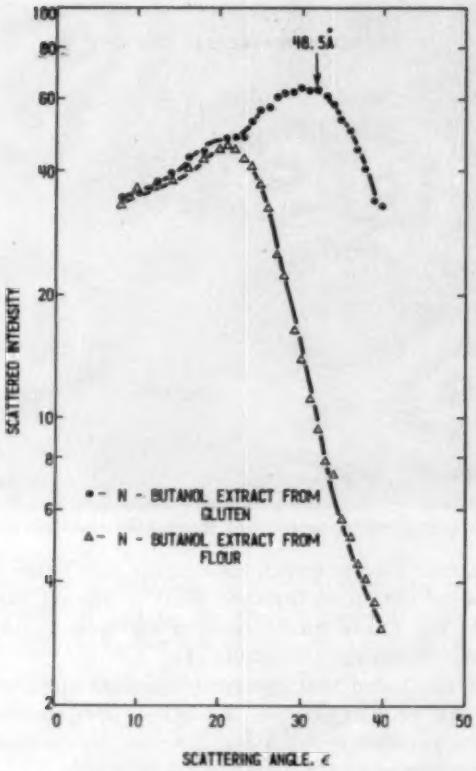

Fig. 7. X-ray scattering at small angles from lipids extracted from flour and developed gluten.

fragile. It appears to be elastic, but with a very low elastic limit, i.e., it ruptures easily. Most important, there is no plasticity. That is, it is not possible to induce large permanent deformations without rupturing. This distinction between elasticity and plasticity in gluten is a necessary one which is not always carefully made. Both properties are necessary for a dough of optimum baking quality.

More sophisticated examination of the lipid-free glutens[8] has shown that the platelet nature of the X-ray scattering persists. That is, scattering curves from lipid-free glutens are identical to those shown in Fig. 1, with the exception of the sharp diffraction peaks. Moreover, it is not possible to develop good gluten sheet structure in stretched,

[8] In the following discussion, all lipid-free samples were extracted after the procedure of Mecham and Mohammad (6).

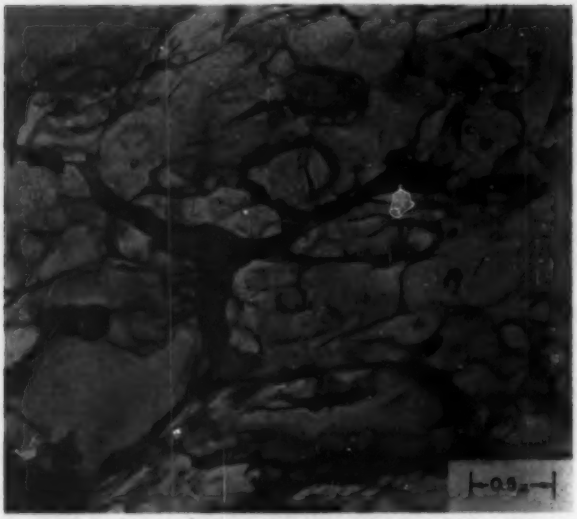

Fig. 8. Surface replica of stretched, freeze-dried, lipid-free gluten.

lipid-free glutens. Two observations support this latter statement. It is not possible to obtain an oriented X-ray scattering pattern such as that shown in Fig. 2, nor do electron micrographs of surface replicas show a well-developed sheet structure (Fig. 8).

It may be concluded that the removal of phospholipids does not destroy the basic protein platelet, but that it does seriously affect the ability of these platelets to bond into coherent sheets capable of large plastic deformation.

Discussion

The Protein Platelet. The experimental evidence discussed in the last paragraph has shown that the platelet structure as detected by X-ray scattering is a property of the protein alone and is not dependent on the presence of lipids. The X-ray data permit a determination of the approximate thickness of the platelet (3), but give no direct evidence as to its lateral extent. However, a comparison of the experimental curve with those calculated theoretically by Schmidt (8) for polydisperse ellipsoids of revolution has shown that the ratio of thickness to diameter is of the order of 0.01.

This protein platelet is most probably formed in the presence of water during the doughing process, and the author assumes that it consists of coiled (perhaps randomly) polypeptide chains with their hydrophilic side chains oriented outward and their hydrophobic side

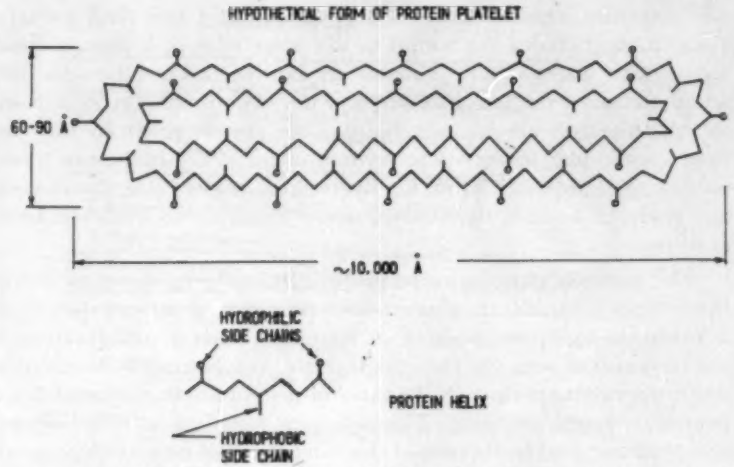

Fig. 9. Proposed model of protein platelet structure.

chains oriented inward. Figure 9 is a hypothetical, highly idealized model of such a protein platelet.

Lipoprotein Linkage between Platelets. Stoeckenius (9) found that mixing pure phospholipids in an aqueous solution with globin produced a lipoprotein complex whose structure was best described by

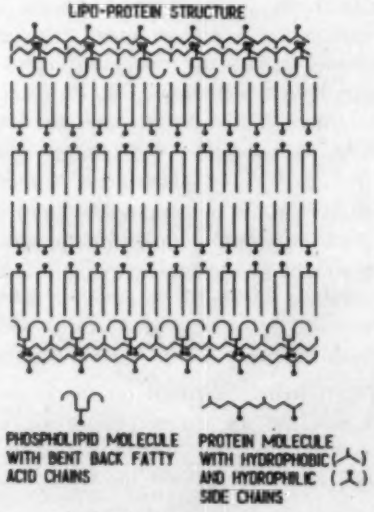

Fig. 10. Lipoprotein structure.

the molecular scheme shown in Fig. 10. At least two (and perhaps more) protein chains are bound to the outer edge of a phospholipid bimolecular leaflet array, probably by saltlike linkages between the acidic groups of the phospholipid and the basic protein groups. Note also that the hydrophobic side chains of the protein penetrate into the lipid bimolecular leaflet—a configuration not easily broken in water solution. On the other hand, the hydrophilic protein side chains stick out ready to bond with other proteins or simply to absorb a layer of water.

The question arises as to the probability of the existence of such a lipoprotein complex in gluten. Several widely separated pieces of information exist, which show, at least, that such a configuration is not inconsistent with the facts. Balls, Hale, and Harris (1) determined the nitrogen-phosphorus (N/P) ratio in a lipoprotein extracted from flour with petroleum ether. This ratio was found to be 8.05:1. Pence and Mecham[4] find N/P ratios of this same order of magnitude in some fractions of the normal butyl alcohol extracts of flour. The α-helix of protein contains 3.6 residues per turn and, hence, an equal number of nitrogen atoms per turn, exclusive of the nitrogen in the side chains. The majority of amino acids found in wheat contain one additional nitrogen atom in the side chains, and hence the total nitrogen per turn of the helix is on the average of the order of 7. Each phospholipid molecule contains one phosphorus atom. Hence, it would not be inconsistent with the N/P ratios quoted above to postulate that a phosphoric acid end group exists for every turn or so of the polypeptide helix. If one counts up the phosphorus atoms per turn depicted in the hypothetical scheme of Fig. 10, there is approximately a 1:1 ratio. Thus, it is not inconsistent with the data at hand to propose that a lipoprotein complex of the type depicted in Fig. 10 exists in gluten.

On the basis of the known percentages of lipid phosphorus found in glutens (6,10), the lipoprotein complex just proposed would amount to about 2½% by weight of the total protein present. If one further assumes that the glutenin fraction is responsible for most of the elastic properties (5,7), then the lipoprotein complex would amount to about 5% of the total elastic structure.

A model of gluten structure based on the evidence at hand may now be proposed. Because the major proportion of protein present in gluten is in platelet form, the continuous sheets observed in the electron micrograph must be formed mostly by the linkage of one

[4] Pence, J. W., and Mecham, D. K. Private communication.

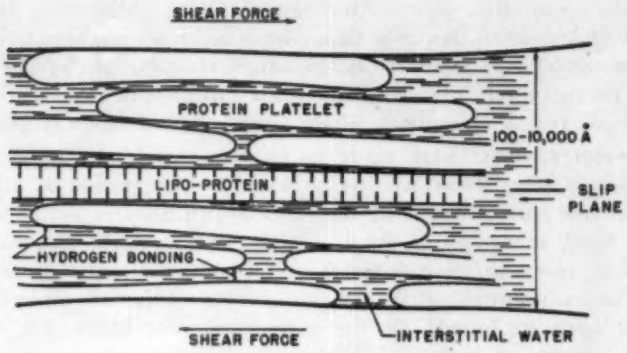

Fig. 11. Proposed model of gluten sheet structure.

platelet with another through an aqueous phase, probably by hydrogen bonding. Five per cent of the elastic sheet, however, must consist of the lipoprotein, which can also bond to the protein platelets through hydrogen or saltlike linkages. Figure 11 is a hypothetical scheme showing a possible structure for a gluten sheet made up of protein platelets and lipoproteins in the appropriate concentrations.

To understand the elastoplastic properties of such a configuration it is necessary to consider the relative bond strengths involved. The hydrogen and saltlike linkages are much stronger than the pseudo bonds existing between the phospholipid bimolecular leaflets, either at the hydrophobic or hydrophilic ends. Moreover, the nature of the two bonds is entirely different. The former are short range and, if broken, unlikely to rebond until the proper chemical groups are brought into close contact again. On the other hand, the bimolecular leaflet configuration results from the dielectric properties of the phospholipid molecule and the pseudo bonds between hydrophobic and hydrophilic ends are fairly long range. Especially is it possible to displace one leaflet with respect to the other in shear as shown in Fig. 11, without disrupting the over-all orientation. Thus, because of the nature and relative strengts of the bonds involved in Fig. 11, an applied stress will result in slip along the interfaces of the phospholipid layers before the rupture strength of the interprotein bonds is reached. This is not to say that rupture will never occur; indeed, the evidence of Fig. 4 shows that rupture does occur frequently. However, on the proposed model, such rupture will occur either in regions poor in lipoproteins or when the plastic strain in the lipoprotein exceeds even the range of the dielectric orienting forces. If the lipoprotein is extracted from gluten the ability to deform plastically (slip) is removed

and the application of stress can result only in widespread rupture of the gluten sheets, once the elastic limit has been reached. It is also easy to see on such a model that too much phospholipid would make for a runny or very plastic gluten, while too little of this component could give rise to a nonplastic gluten easily ruptured under large stress.

Several attempts were made to confirm this model by electron microscopy, using the techniques employed by Stoeckenius (9). Samples of stretched gluten were fixed and stained with osmium tetroxide and then dried in successive ethyl alcohol washes. Since the osmium tetroxide reacts at unsaturated double bonds, it will be distributed more or less uniformly along the protein chain (wherever unsaturated amino acids are found). On the other hand, Stoeckenius has shown that it will deposit preferentially along the interior of the phospholipid bimolecular leaflet, i.e., at the hydrophobic ends. Because the osmium is highly opaque to electrons, the bimolecular leaflets should then appear as alternating light and dark lines in the microscope, separated by approximately 40 to 50 angstrom units. The attempts which we have made to detect these striations have not been successful. Because we expect at most only 5% of a field of view, say, to consist of these lines, it is very easy for artifacts to be misinterpreted as the true structure. Secondly, 40A is near the limit of resolution of the electron microscope used in these studies. Although striations of the correct spacing have been observed on occasion, the difficulties just mentioned do not allow one to interpret this as an unqualified confirmation of the proposed structure.

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MICROTESTS FOR FLOUR QUALITY[1]

H. R. ELLING AND M. A. BARMORE[2]

ABSTRACT

Flour quality characteristics are predicted from tests using 0.4 g. of the coarse flour available from a 5-g. micromill. Strength, i.e., loaf volume and cookie diameter, are predicted from the relation of the gas-retention properties of a fermenting dough made from the flour extracted with a dilute sodium chloride-potassium bromate solution compared to that made from the unextracted flour. Mixing time is determined on a grooved spindle rotating in a close-fitting glass tube. Absorption is determined from the water retained, after centrifuging, in the flour extraction procedure. The predictions are based on the highly significant correlations of the results of the microtests with those obtained from well-known varieties by conventional experimental baking laboratory methods.

Recently this laboratory developed a 5-g. mill (8,9) for use in wheat breeding programs in which an improvement in milling quality was one of the objectives. Such early screening enables the wheat breeder to concentrate his performance tests on strains of suitable milling types. The flour obtained from milling 5 g. of wheat on this mill resembles a coarse middlings stock, as it passes through a 38-wire sieve, and contains some bran particles. The purpose of the work described in the present paper was to develop tests which could be used to predict the baking quality from the 2 to 3 g. of flour obtained from milling a 5-g. sample of wheat.

The nature of the flour and the amount available per sample were limiting factors in determining the type of tests to be developed. Pre-

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liminary results on the meal indicated that lactic acid sedimentation (Zeleny's test, 7,11) and viscosity measurements (apparent acid viscosity, 1) would be of little value. The granularity of the meal and the relative high ash value apparently made measurements of this nature meaningless. The alkaline water-retention capacity (AWRC, Yamazaki, 10) was satisfactory for samples between the two extremes of good and poor cookie diameter. Since practically the same information could be obtained by determining the water absorption of the sample, the AWRC test was not pursued further.

One of the most important quality properties of flour is its ability to produce a dough that has good gas retention. Various methods have been described for determining this property (4,5,6). Varying degrees of success have been reported. Miller *et al.* (6), working with spring wheat, found a correlation of 0.81 to 0.90 between gas retention and loaf volume. Harris (4), working with North Dakota and Mexican selections, found a low correlation. These results indicated that reliable differentiation between samples of one type of wheat was obtained, but differentiation was poor when several types were included. In spite of this situation and the fact that the Pacific Northwest wheat breeders have used all types of wheat in their work, gas-retention investigations were undertaken.

Materials and Methods

The 228 wheat samples used consisted of 24 varieties commonly grown in the Pacific Northwest and were from the 1952 through 1958 crops. To illustrate the characteristics of the samples studied, quality data of the maximum and minimum protein levels of four of the 24 varieties are given in Table I. These data are typical and represent the range in properties of the other varieties. The weakest was Brevor, and the strongest was Itana. Since all of these samples had been

TABLE I
QUALITY DATA FOR TYPICAL VARIETIES

Variety	Market Class*	Flour Protein	Loaf Volume	Cookie Diameter	Water Absorption	Mixing Time
		%	*ml*	*cm*	%	*minutes*
Brevor	SW	5.4	610	9.11	55.0	4.20
		11.4	873	...	65.0	2.50
Idaed	SW	6.7	700	9.00	53.0	3.70
		13.7	1,035	...	68.0	3.80
Kharkof	HRW	5.5	625	8.34	61.0	3.60
		13.2	980	...	75.0	3.90
Itana	HRW	7.5	...	7.91	65.0	...
		12.9	910	8.25	67.0	7.75

* SW, soft white; HRW, hard red winter.

tested for baking quality by conventional experimental milling and baking methods (2), detailed quality data were available for comparison with results from the microtests. The meal used for development of the tests was obtained from the 5-g. mill by Seeborg and Barmore's method (8). The following solutions were used:

1) Dilute salt solution: 0.5% sodium chloride plus 0.05% potassium bromate;
2) Yeast solution: 1.5 g. dried yeast in 10 ml. distilled water which had been standing at room temperature for 12 hours;
3) Concentrated glucose-salt solution: 61 g. anhydrous glucose and 2.4 g. sodium chloride in 100 ml. water; and
4) Dilute glucose-salt solution: 6 g. glucose and 0.5 g. sodium chloride in 100 ml. of water.

The following methods were developed for 0.40 g. of meal:

Dough Method. To the 0.40 g. of meal in a 12-mm. glass test tube was added 0.1 ml. of the yeast solution, 0.06 ml. of glucose-salt-bromate solution, and 0.722 times the water retained in the extraction method less 0.110 ml. The meal was mixed with a stiff wire until wet, allowed to stand for 15 minutes, removed from the tube, kneaded six times, rolled ten times between the palms of the hands, and placed, partially flattened, in the mouth of the mixer tube. The mixing tube and dough were placed over the rotating mixing shaft, and the dough was mixed until it smeared against the side of the tube or wrapped itself around the shaft. During the mixing, the tube was moved back and forth over the mixing shaft about 2 in. each second in order to keep the diameter of the dough relatively constant. Following mixing, the dough was removed from the tube and shaft, kneaded six times, rolled ten times, and put into the high-humidity fermentation cabinet at 37.5°C. for 45 minutes. From this point the extracted-dough procedures were used.

Extracted Flour Dough Method. The 0.40 g. of meal was placed in a preweighed polyethylene centrifuge tube of 6 ml. capacity and 1 by 10 cm. i.d.; 3.5 ml. of dilute salt solution were added; the tube was stoppered, and shaken vigorously on a wrist-action mechanical shaker for 15 minutes to disperse the meal. To this were added 0.1 ml. yeast and 1.0 ml. of the concentrated glucose-salt solutions, followed by hand shaking, centrifuging for 20 minutes (at 5,050 relative centrifugal force), decanting, draining for 15 minutes inclined at an angle of about 15° and weighing. The increase in weight was called E.H_2O, although it is obvious that some material was lost in the extraction and some weight gained because of yeast cells. The wet

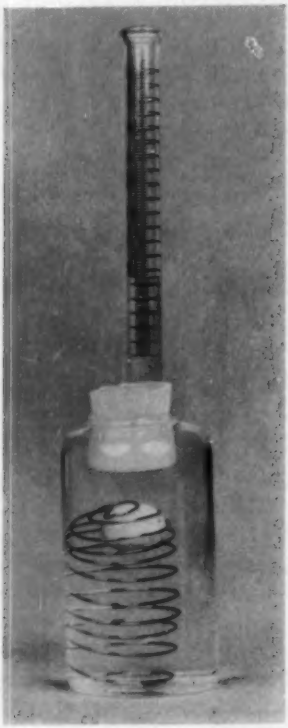

Fig. 1. Gas-retention or Paley bottle with dough and cage in place.

mass was mixed in the tube for 40 seconds, using the motor of the micro mixer and a shaft of 1/8-in. key stock. The dough was then removed from the tube, kneaded, rolled, and placed in the fermentation cabinet.

Gas Retention. Gas retention was measured in a Paley bottle (Fig. 1), which is a flask used to measure the fat content of cheese or ice cream. A cage was developed and inserted in the bottles to hold the dough away from the outlet. The bottles were filled to the zero level of the graduated stem with the dilute glucose-salt solution and held in a water bath at 45°C. The fermented dough was inserted under the cage and allowed to expand in the dilute sugar-salt solution to its maximum size. Gas released from the dough escaped from the bottle and was not measured.

Absorption. Absorption was designated as the amount of water required to produce a soft, pliable dough as determined by ap-

pearance and feel during the hand-doughing procedure. Estimates of the amount of water to use were taken from the water retained by the flour in the extraction step ($E.H_2O$).

Optimum Mixing Time and Micro Mixer. The optimum mixing time was determined in a micromixer on the fermented dough in the dough method. This mixer consists of a motor-driven shaft 8 in. long, 0.35 in. in diameter (the last 4 in. tapered from 0.35 to 0.30 in.), operating in a glass tube of 0.45 in. i.d. at about 280 r.p.m. The shaft has a coarse spiral groove cut in it. The small end has a right-hand and the motor end a left-hand groove; this groove tends to keep the dough from stretching along the shaft in an excessive amount. Optimum mixing was the time required to mix the dough until it began to smear on the glass tube.

Results

Data were recorded on optimum micro mixing time, maximum expansion volume by the dough method (DV), maximum expansion volume by the extracted flour method (EV), the amount of water retained by the flour in centrifuging ($E.H_2O$), and the amount of water required to produce a soft, pliable dough ($D.H_2O$). Table II illustrates the degree of association obtained.

From these data, it is obvious that baking absorption, loaf volume, and optimum mixing time can be predicted with considerable reliability, and cookie diameter with somewhat less certainty.

It was found that EV was significantly correlated with DV for 20 of the 24 varieties. Four of the varieties are shown in Fig. 2 as examples. The "t" test showed that many of the regression coefficients were significantly different. Over the entire range, which varied from

TABLE II

SIMPLE CORRELATION COEFFICIENTS AND STANDARD DEVIATION OF ESTIMATE FROM REGRESSION

MICRO QUALITY OBSERVATIONS	EXPERIMENTAL BAKING OBSERVATION	CORRELATION COEFFICIENT	STANDARD DEVIATION OF ESTIMATE
$D.H_2O$	vs. 100-g. bread-baking optimum absorption	0.95**	1.60%
$E.H_2O$	vs. 100-g. bread-baking optimum absorption	0.90**	2.19%
$D.H_2O$	vs. cookie diameter	—0.77**	0.28 cm.
$E.H_2O$	vs. cookie diameter	—0.73**	0.30 cm.
$E.H_2O$	vs. AWRC	0.73**	4.9%
EV	vs. loaf volume	0.71**	96 ml.
DV	vs. loaf volume	0.84**	75 ml.
(EV + DV)	vs. loaf volume	0.95**	42 ml.
Optimum micro mixing time	vs. 100-g. bread-baking optimum mixing time	0.94**	0.57 minutes

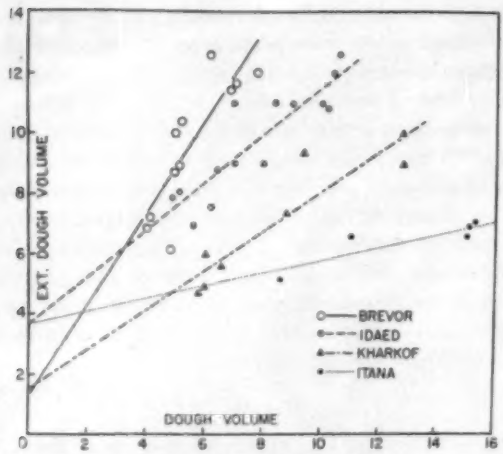

Fig. 2. Relation of extracted (EV) vs. dough (DV) expansion volumes.

1.97 to 0.05, the regression coefficients could be divided into three groups, the extremes of which were significantly different at the 3% level or lower. In Fig. 2, the coefficient for Brevor was significantly different from that of Idaed, and Kharkof from Itana, at the 3% level. That for Idaed was not significantly different from Kharkof, as would be expected from inspection. Thus it was concluded that the regression coefficients characterized approximately the varieties — at least 20 of the 24.

The magnitude of the EV and DV values was significantly influenced by the protein content of the flour, as indicated by the multiple correlation coefficient of 0.945** for EV and DV vs. protein content. This conclusion is also evident from the correlations between protein content and both EV and DV for each variety. These coefficients averaged 0.87 and 0.92, respectively. Thus, as protein content increased for samples of the same variety, the EV, DV values increased proportionally. Since there was a significant correlation between the regression coefficients of the 20 varieties with significant DV vs. EV correlations and the values of EV at a mean value of DV, the equation of a family of lines was determined by a method previously described (2,3), which best represented these 20 varieties. In the light of this generalization, the assumption was made that this same family of lines will represent data obtained from new wheat hybrids. Consequently, EV,DV data on new selections located on this family of lines should indicate their general properties, i.e. their strength. The

results indicate that unknown selections can be classified as to their relative strength regardless of protein level; in fact, protein content can be estimated from the multiple regression equation of EV = 2.287 − 0.839 DV + 1.426 protein content.

In the application of the method to the characterization of new selections, The EV and DV values are plotted as in Fig. 2. The line drawn from the EV,DV point to the common origin (DV = 2.2 and EV = 3.6) subtends an angle with the DV axis that characterizes the new selection; i.e., this angle indicates that the new selection will have flour properties similar to those of a commercial or known variety that yielded a similar angle by the same methods.

One technician can run about 24 samples in an 8-hour day, determining absorption, mixing time, and extracted and unextracted flour dough expansion volumes.

The reason for the relative difference in gas-retention values obtained by the two methods is unknown. It will be noted that the extracted flour doughs give higher gas-retention values for the weak varieties than for the strong varieties; conversely, the unextracted flour doughs give higher gas-retention values for the strong varieties than for the weak ones. Brevor was the weakest variety and Itana was the strongest type. The locations of the EV,DV values in Fig. 2 illustrate the relation of these values to flour strength. The two methods differ only by the extraction procedure used in obtaining the extracted dough gas-retention value. Therefore, one of the factors in wheat flour responsible for its quality must be located in the soluble portion of the flour. In the weak varieties, this fraction reduces the ability of the proteins to retain gas, causing gas-retention values of the unextracted flour to be lower than that obtained when the fraction is removed. For the strong types, the reverse is true. The soluble fraction, being beneficial to the retention of gas, causes the unextracted flour to give higher values than the extracted flour. A study is under way to find the reasons for this difference, because it is believed a much improved micromethod could be developed if that information were available.

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THE FORMATION OF CRACKS IN RICE DURING WETTING AND ITS EFFECT ON THE COOKING CHARACTERISTICS OF THE CEREAL[1]

H. S. R. DESIKACHAR AND V. SUBRAHMANYAN

Milled raw or parboiled rice developed transverse lines of cracks when soaked in water. It took a longer time for the cracks to develop in parboiled rice than in raw rice. Formation of cracks was accelerated by temperature in the case of parboiled rice; whereas a retarding effect was observed above 70°C. in raw rice.

After grains were soaked in water to develop these cracks, both raw and parboiled rice required a shorter cooking time, and the cooked grains were also longer than in the case of the unsoaked control samples.

The absorption of water by rice grains made them opaque, and this property was used to study the mode of water penetration during soaking. In the initial stages, water entered the grain near its germ end and along the top line of fusion. Transverse lines of cracks developed after some time, and water entered the grain through these cracks subsequently. If the grains were already cracked before wetting, water absorption was quite fast, and the grains became opaque within a few minutes.

The development of checkings or cracks in rice and other grains during dehydration as a result of quick or uneven removal of water is well known, but the occurrence of similar cracks during hydration or wetting is very little understood. Rice is very susceptible to this type of cracking during moistening or wetting (3), and the object of the studies reported here is to investigate this phenomenon and to study its effect on the cooking property of the rice.

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Initial studies revealed that the development of these cracks was dependent on time and the temperature of the soak water, and, hence, the progressive development of these cracks at different temperatures in both raw and parboiled rice was first studied. Rice grains which develop cracks during milling or dehydration are known to break and cook to a pasty mass and yield a viscous gruel; it was, therefore, thought that cracks formed during wetting or soaking of rice in water may also cause the rice to cook poorly. The effect of wetting on the cooking properties of the rice was, therefore, studied. Attempts were also made to see whether the study could throw some light on the mode of water penetration during soaking in water.

Materials and Methods

Data have been presented here on two varieties: Ratnachudi (soft medium grain) and Bangarasanna (hard fine grain) as representative of about six varieties on which observations were made. The paddy samples were parboiled by soaking in water at 70°C. for 3 hours, draining off the soak water, steaming for 15 minutes, and drying in the sun. The parboiled and raw samples of paddy were milled thoroughly to remove the bran and sieved to remove broken grains.

For studying the effect of time and temperature on the development of cracks, ten sound grains free from visible cracks or other defects were suspended in water maintained at the desired temperature, and the cracks or striations appearing on the surface of the grains were counted at different periods. The appearance of the cracks was best seen by observing the grains under water in a beaker and illuminated from beneath. Under such transmitted light the cracks appeared as dark lines on the grain. Raw and parboiled rice of Bangarasanna variety was used for these studies.

From the above experiments, it was found that parboiled rice required 45 minutes to develop cracks to the maximum extent; whereas, in the case of raw rice, a similar degree of cracking developed within 15 minutes. Hence, for the cooking studies, 25-g. samples of the raw and parboiled rice were soaked in water for 15 and 45 minutes, respectively, for full development of cracks, and then cooked in 150 ml. boiling water; the cooking time and the loss of rice solids in the cooking water (gruel) were measured. Unsoaked control samples of rice and also raw and parboiled rice samples, soaked only for such period as would not allow cracks to develop (about 4 and 15 minutes for raw and parboiled rice, respectively), were also cooked under similar conditions for comparison. The minimum time needed for the rice grains to cook to a soft consistency (as pressed between two

glass plates) without presenting a core of hard opaque starch was taken as the cooking time. The excess cooking water was drained through a strainer, evaporated to dryness first over a water bath and then to constant weight in an oven at 110°C., and weighed. Since differences between the two treatments (soaked and unsoaked) in the size and shape of cooked grains could be seen visually (Fig. 1), the average length and breadth of the raw and parboiled cooked grains were measured using the method described earlier (1).

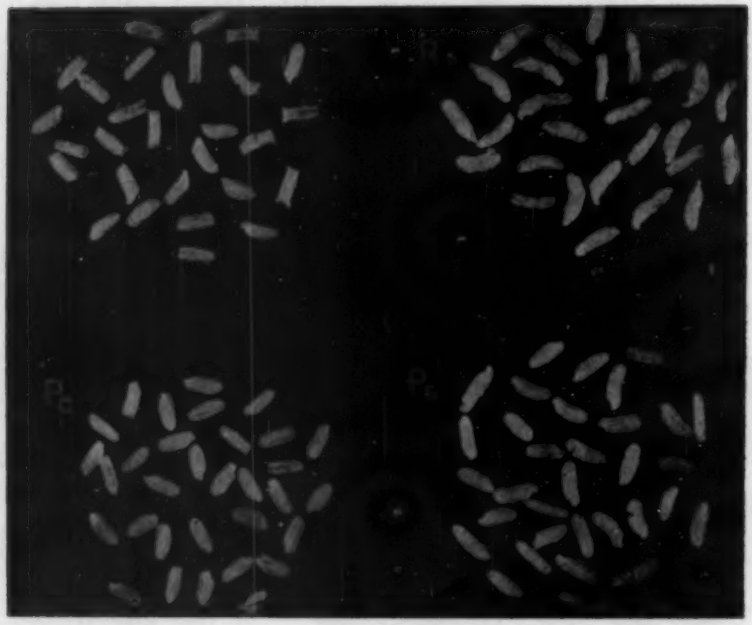

Fig. 1. Effect of presoaking rice in water prior to cooking on the appearance of cooked grains. c, control (unsoaked); s, soaked; R, raw; P, parboiled.

In the course of the above studies, it was observed that the absorption of water during soaking of rice grains, which are normally flinty or translucent, made them opaque. This fact suggested a method for studying the mode and extent of water penetration in rice during wetting. Initial experiments indicated that the top (dorsal) line of fusion may be the line at which water penetrates the grains. Tests were, therefore, carried out on select sound grains, along with grains with visible surface cracks for comparison, to observe the extent of water penetration. Photographs of the grains were taken at short intervals during soaking until the whole grain was opaque.

Results and Discussion

Development of Cracks during Soaking. The development of cracks on the grains immersed in water seems to be a direct effect of hydration, since the development of these lines is a gradual process and depends upon the temperature of the soak water (Figs. 2 and 3). At ordinary temperatures (30°C.), a certain time (about 3 or 4 minutes in the case of raw rice and about 20 minutes in the case of parboiled rice) elapses before the cracking starts, which suggests that hydration precedes cracking. Parboiled rice requires a longer time to develop cracks because it is less permeable to water and has a lower rate of hydration.

The effect of temperature generally is to hasten the water imbibition by both the raw and parboiled rice, and hence the initial lag period before the grains start cracking is shortened. The time taken for maximum development of cracks is also reduced at high temperature. It can be seen from Fig. 2 that the lag period of parboiled rice at 30°C. is 20 minutes; it is reduced to about 5 minutes at 90°C. In raw rice the cracking process is hastened as the temperature is increased to 60°–70°C. At higher temperatures the number of cracks become fewer, and this is especially marked at 90°C. The few cracks become cemented within a short period because of the rapid gelatinization of the starch.

Cracks in Relation to Cooking Characteristics of the Rice. The effect of the cracks on the cooking quality of the rice is shown in Table I. Since the cracks permit entry for water into the rice grain, the cooking time is reduced by presoaking the grains for a sufficient

TABLE I

EFFECT OF PRESOAKING RICE IN WATER ON ITS COOKING CHARACTERISTICS

RICE VARIETY	TIME OF SOAKING	TIME NEEDED FOR COOKING	LOSS OF SOLIDS IN COOKING WATER	AVERAGE DIMENSIONS OF COOKED GRAINS[1]	
				Length	Breadth
	minutes	*minutes*	%	mm	mm
Ratnachudi (raw)	15	12	8.2	9.73 ± 0.51^{a}	3.05 ± 0.16
	4	19	8.6	....	....
	0	20	9.0	$7.98 \pm 0.44^{a'}$	3.01 ± 0.16
Bangarasanna (raw)	15	13	9.0	9.79 ± 0.65^{b}	2.76 ± 0.16
	4	21	8.8	....	....
	0	21	10.8	$7.73 \pm 0.36^{b'}$	2.79 ± 0.20
Ratnachudi (parboiled)	60	15	4.8	9.39 ± 0.91^{c}	3.15 ± 0.16
	15	22	4.9	....	....
	0	25	5.1	$7.83 \pm 0.44^{c'}$	3.38 ± 0.20

[1] The average lengths and breadths respectively of the uncooked grains were 5.25 ± 0.07 and 1.96 ± 0.04 for Ratnachudi (raw); 5.46 ± 0.07 and 1.67 ± 0.04 for Bangarasanna (raw); and 5.22 ± 0.07 and 1.99 ± 0.02 for Ratnachudi (parboiled) varieties of paddy.

The differences between a and a'; b and b'; c and c' are highly significant ($P < 0.001$).

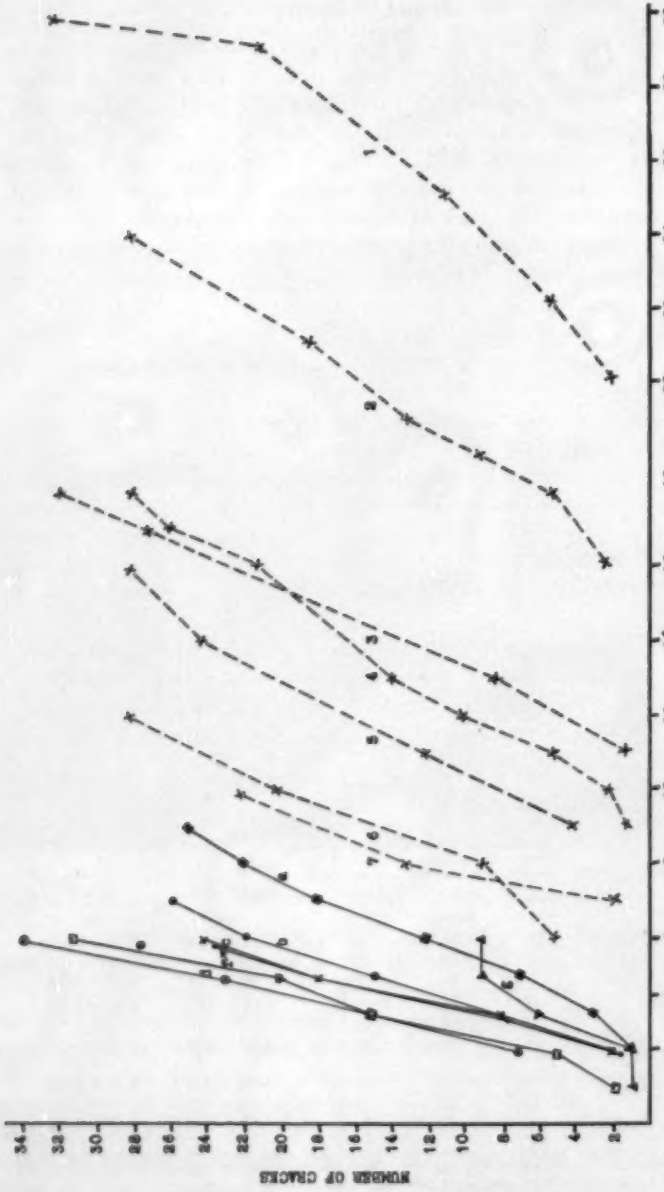

Fig. 2. The effect of time and temperature on the formation of cracks in rice during wetting. 1, 2, 3, 4, 5, 6, and 7 and a, b, c, d, e, f, and g represent curves for parboiled and raw rice, respectively, at 30°, 40°, 50°, 60°, 70°, 80°, and 90°C.

period of time to develop cracks. The effect of wetting *per se* (without the development of cracks) was negligible. The cooking time for these samples was very nearly the same as for the unsoaked control samples. It was feared that the development of cracks might increase the leaching of rice solids into the cooking water. This has not, however, been found to be the case. The reduction in cooking time, by providing points of entry for water, has brought the loss of solids in the cooking water (gruel) to levels either equal to or less than that for the control samples.

The effect of soaking on the general appearance of cooked raw and parboiled grains is presented in Fig. 1. The soaked grains, on cooking, were considerably longer than those cooked without presoaking, although the differences in the breadth of the cooked grains were not significant (Table I). Water entered the grain through the transverse lines of cleavage and caused greater expansion of the grain along the length. (See below for mode of water entry through cracks.) When the rice was put directly into boiling water, the formation of cracks was minimized, and the cooking time was longer.

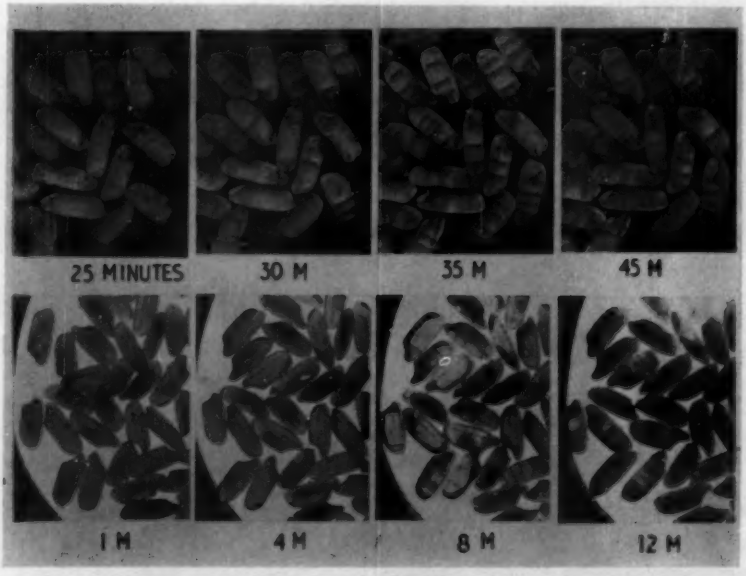

Fig. 3. Rice grains at different stages of wetting. Top row, parboiled (photographed by reflected light); bottom row, raw grains (by transmitted light). The longitudinal striations on the raw rice grains, which are visible both before and after treatment, represent lines of bran not removed during milling.

Mode of Water Penetration during Soaking. When raw rice grains immersed in water were observed under a microscope with low power (×20) it was seen that a dark line first developed near the germ tip and along the dorsal line of fusion. This later spread inward like a shadow, and, after a time, transverse cracks developed as dark lines. These lines then broadened into dark bands. The band or shadow increased in area with increased time of soaking. It appeared that the dark lines or shadows represented portions wetted by the water. Actual photographs taken through transmitted light (bottom illumination) have confirmed the above view (Fig. 3). This can be very clearly seen in the raw rice. The white islands, or pockets, in the endosperm represent portions not wetted by the water.

Figure 4 presents photographs (by reflected light) of raw rice grains with progressive wetting. Examination of the photographs shows that water began to enter the sound grain at points near the germ end, and that entry was along the top line of fusion. The area of the white (opaque) region gradually extended inward until cracks developed, and, thereafter, the water movement was on both sides of the transverse lines of cracking. In grains which had slight cracks before soaking in water, the pattern was similar, but the entry of water

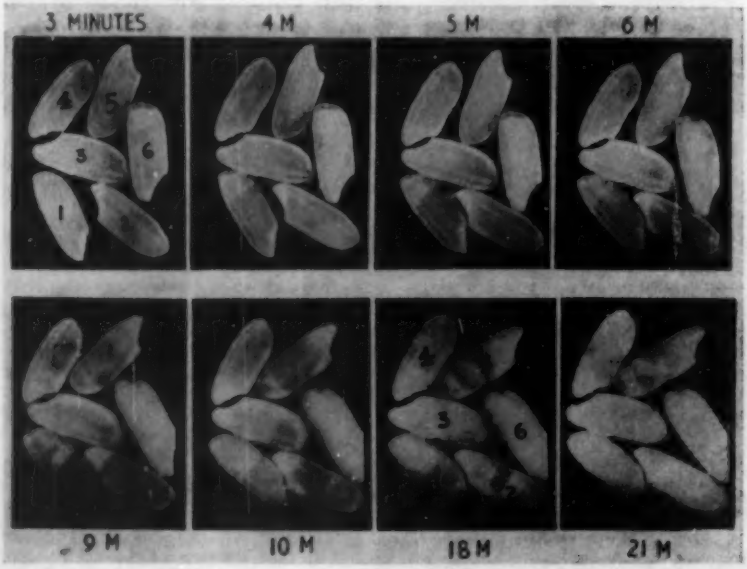

Fig. 4. Penetration of water in raw rice grains. Grains 3, 4, 5: sound grains without cracks; grains 1 and 2: with slight cracks; grain 6: with extensive cracks.

through the cracks was fast, and the grains became opaque much earlier than did the sound grains. In the case of the grain with extensive visible cracks, the whole grain became opaque in about 5 minutes. The pattern presented by parboiled rice was of the same type although the results were not quite as clear (Fig. 5). Even when visible cracks were present, the rate of absorption of water, as judged by the increase in the opaque surface, was slow. There is also some evidence that the top line of fusion became cemented in grain 1 as a result of full gelatinization during parboiling. In other grains, however, the entry of water was, as in the case of raw rice, mostly along the line of fusion. The rate of entry was, however, very slow.

Another feature of soaking is the accumulation of gas bubbles around the grains. These bubbles increase in size and number during the soaking and are not, therefore, due entirely to the film of air around the surface of the grain before it is put into water. Presumably, the entry of water displaces the air inside the grain.

A fundamental aspect which needs to be pursued is the reason for the development of cracks during wetting and the orientation of these cracks mostly parallel to the short axis (breadth) of the grain. Whether the cracking is due to stresses set up by differential hydration of the

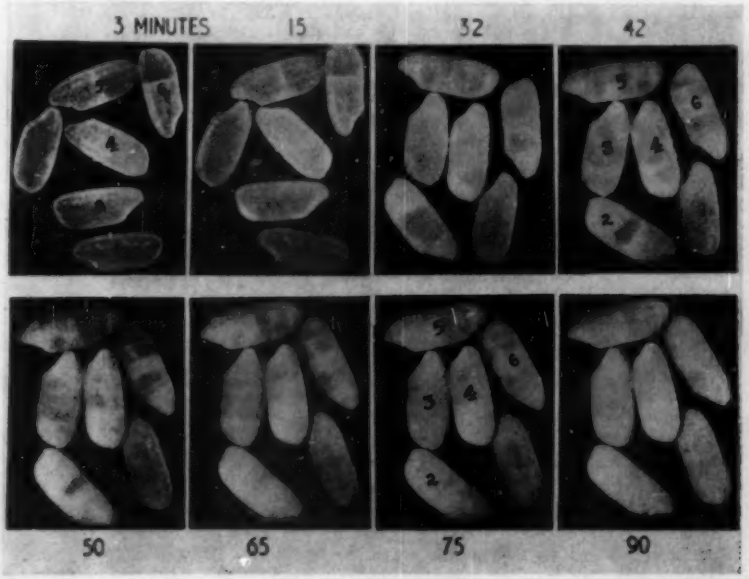

Fig. 5. Penetration of water in parboiled rice grains. Grains 1, 2, 3, 4: sound grains free from cracks; grains 5 and 6: with visible surface crack before wetting.

different constituents or release of internal stresses already existing inside the grain, as has been postulated in the case of wheat (2), is to be further investigated. It is also proposed to study the water penetration in paddy where the rate of water movement would be slow. The development of cracks, if any, in such a case has to be observed by X-ray photographs.

Acknowledgment

The authors wish to thank Mr. K. V. Achyutha Rao for the photographs.

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HARDNESS AND MOISTURE CONTENT OF WHEAT KERNELS[1]

R. Katz, N. D. Collins, and A. B. Cardwell

ABSTRACT

The hardness of kernel sections of hard red winter and soft white winter wheat and durum wheat decreased with increasing moisture content. Small variations in hardness with variety and test plot location were detected.

The hardness of samples of several varieties of hard red and soft white winter wheat and of durum wheat, grown in crop years 1956 and 1957 in test plots in Kansas and North Dakota, was determined in relation to moisture content by means of a grain hardness tester recently developed at this laboratory (2) from a commercial hardness tester called the Barcol Impressor. In the present investigation Model II of the tester was used. This model was provided with two testing ranges, a high range used for testing hard wheat and a low range used for testing soft wheat or hard wheat at high moisture content.

Materials and Methods

The hard red winter wheat varieties used in this work were Ponca,

[1] Manuscript received March 12, 1960. Contribution No. 76, Department of Physics, Kansas Agricultural Experiment Station, Manhattan.

Kiowa, and Wichita, grown in test plots at Manhattan, Hays, and Belleville, Kansas, in 1957. Durum varieties tested were Mindum, Vernon, and Langdon, all grown at Minot, North Dakota, in 1956. Elmar and Brevor, grown at Manhattan, Kansas, in 1956, were the varieties of soft white winter wheat used.

Following the procedure described by Katz *et al.* (2) transverse sections of wheat kernels, about 1 mm. thick, were taken from the central portion of the kernel to be tested, by use of a freezing microtome, and were cemented to glass microscope slides with Duco cement. Cracked sections, or those with skewed surfaces, were culled from the test samples through visual examination.

To ascertain whether freezing and thawing of the wheat kernels associated with use of the freezing microtome had any effect on kernel hardness, 40 kernel sections were prepared, 20 with the freezing microtome, and 20 from the same lot of durum wheat by using wax to secure them to the microtome stage during sectioning. Both groups were kept at 81% relative humidity. After 1 week one section from each group was tested every day for 20 days. No difference in hardness of the two groups was detected. It was therefore concluded that freezing and thawing on the microtome stage did not affect hardness measurements significantly.

After the Duco cement holding the sectioned kernels had set, the mounted sections were placed in a chamber containing a solution of sulfuric acid in water, of a concentration appropriate to a desired humidity (3). The hardness of kernel sections was measured after moisture equilibrium was reached at eleven different values of relative humidity, from 10 to 95%, at a laboratory temperature of 25°C. To ensure that specimens had reached equilibrium with the chamber atmosphere, a 20-g. sample of the variety being tested was placed in the humidity chamber and weighed on successive days until constant weight was reached. The time to reach moisture equilibrium varied from 10 to 18 days, the extremes of humidity requiring the longest time. When equilibrium had been reached, the 20-g. samples were analyzed for moisture content. The kernel sections were removed from the humidity chamber and examined microscopically for evidence of mold growth. Mold growth was prevented in the 95%-humidity chamber by placing an open dish of toluene in the chamber.

As indicated in the previous article (2), testing was done by placing the glass slide on the micrometer stage of the hardness tester and pressing down on the framework of the tester until the flat part of the tester spindle was in contact with the specimen, at which time the dial reading reached a constant maximum value. All kernels

sufficiently hard for use of the II-H scale were measured at five different points on the kernel section; at high humidities and for soft wheat, where the II-S scale was used, three or four measurements were made on a kernel section. The II-S scale was used for all measurements on the soft white winter wheats, but both scales were used for testing the hard wheats. The use of both scales for testing one sample of wheat did not affect the results, since the two scales were related monotonically; that is, a decrease in hardness on one scale was always associated with a decrease in hardness on the other scale. The relationship between II-S and II-H hardness numbers was a linear one (2).

Hardness measurements were made on nine sections of each sample at each moisture condition. In all cases the average of the readings for a kernel was taken as representative of the kernel section. For convenience in presenting the data, hardness numbers obtained on the II-S scale with hard wheats at high humidity were converted to II-H numbers by using the relationship between the scales (2).

Results and Discussion

Hardness of hard wheat varieties (hard red winter and durum) diminished regularly with increasing moisture content. Soft white winter wheats showed no significant change in hardness up to a moisture content of 13%. Above this moisture content their hardness showed a rapid decrease. In all cases the kernel-to-kernel variation in hardness was much greater at high moisture content than at low moisture content. Durum wheat kernels were the most uniform;

TABLE I

HARDNESS OF VERNON AND LANGDON KERNELS AS A FUNCTION OF MOISTURE CONTENT

AMBIENT HUMIDITY (RELATIVE)	VERNON		LANGDON	
	Moisture	Hardness	Moisture	Hardness
	%		%	
12	6.74	93.2 ± 1.0	5.64	92.7 ± 1.8
22	7.47	92.8 ± 0.4	7.41	93.8 ± 1.2
31	8.56	92.4 ± 0.7	8.43	91.8 ± 0.9
42	10.19	92.9 ± 0.9	9.14	89.6 ± 1.7
50	10.99	91.0 ± 1.1	10.58	86.9 ± 2.3
59	11.94	90.8 ± 0.8	11.42	87.6 ± 1.7
70	13.38	88.7 ± 1.4	13.00	85.3 ± 2.6
80	15.15	*86.8 ± 2.0	14.86	*83.0 ± 3.5
85	16.51	*83.3 ± 1.5	16.13	*80.0 ± 3.0
89	17.97	*71.2 ± 5.3	17.36	*71.5 ± 3.5
94	21.20	*66.1 ± 5.0	20.37	*70.5 ± 4.5

* Hardness was measured with II-S scale and converted to II-H scale.

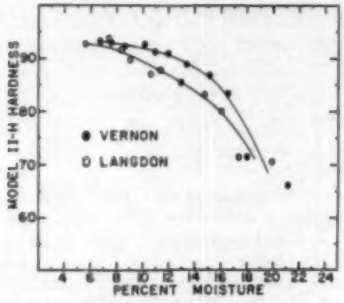

Fig. 1. Hardness of durum varieties Vernon and Langdon as a function of moisture content at 25°C. Standard deviations in the hardness number at moisture contents below 12% were about ± 1.5. Above 12% moisture content the standard deviation increased with increasing moisture from ± 1.5 to ± 5.0.

soft white winter kernels were the least uniform.

While all varieties of durum wheat were more uniform in hardness than other types of wheat, Mindum and Vernon kernels were consistently harder than Langdon kernels in the range of 10 to 17% moisture content. The hardness of Vernon and Langdon kernels as a function of moisture content is shown in Fig. 1 and Table I. Mindum and Vernon kernels were of essentially the same hardness at corresponding moisture content.

Similar varietal differences in hardness were displayed by the soft white winter varieties Elmar and Brevor. Brevor was consistently softer, as shown in Fig. 2, the difference being greatest at higher moisture contents.

No differences in hardness due to variety or test plot location

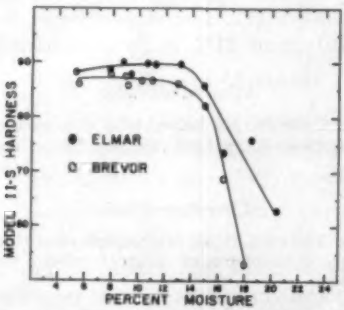

Fig. 2. Hardness of two varieties (Elmar and Brevor) of soft white winter wheat. Standard deviations in the hardness number at moisture contents below 12% were about ± 3.5. Above 12% moisture the standard deviation increased with increasing moisture from ± 3.5 to ± 11.0.

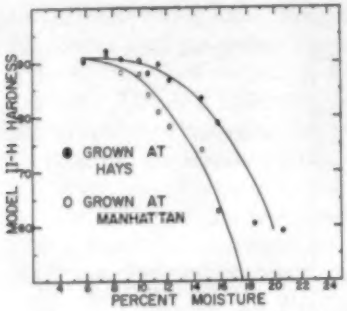

Fig. 3. Hardness of Wichita wheat (hard red winter) grown at Hays and Manhattan, Kansas. At a moisture content of 18.5%, the sample grown at Manhattan had a hardness of 38.5. This point is not shown on the graph. Standard deviations in the hardness number at moisture contents below 12% were about ± 3.5. Above 12% moisture content, the standard deviation increased with increasing moisture from ± 4.0 to ± 10.0.

were found in the hard red winter varieties Ponca or Kiowa grown in Belleville, Hays, and Manhattan, Kansas. At the higher moisture contents some differences were found in the hardness of Wichita wheat, samples from Manhattan being somewhat softer than samples raised at Hays or Belleville. The hardness of Wichita, grown at Manhattan, and Hays, as a function of moisture content, is shown in Fig. 3.

The hardness of hard red winter wheat was nearly equal to that of durum wheat at moisture contents below 13%, but at high moisture contents the durum wheats were generally harder.

For all wheat tested, the relationship between ambient humidity and moisture content at equilibrium at 25°C. agreed with the results of Coleman and Fellows (1). The equilibrium moisture was 6% at 10% relative humidity, and 21% at 95% relative humidity.

Acknowledgments

M. Milner and J. A. Shellenberger helped plan this research and provided suitable samples for investigation; J. Spangler and G. Urban assisted with a number of the measurements.

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EFFECT OF BLEACHING ON FLOUR AS MEASURED BY STRUCTURAL RELAXATION OF DOUGH[1]

ENDEL J. JASKA[2]

ABSTRACT

Differences in structural relaxation of a hard red spring wheat flour dough were measurable when bleached with 3.3 and 5.5 p.p.m. of chlorine dioxide in the presence of 200 p.p.m. benzoyl peroxide. The increase of the relaxation hyperbola semiaxis constant caused by increased chlorine dioxide concentration and measured at a 3-hour reaction time was approximately constant for doughs mixed in air for 2.5 minutes and 10 minutes. The increase of the semiaxis constant caused by the farinograph mixing time difference of 2.5 minutes and 10 minutes was also approximately constant at different concentrations of chlorine dioxide. The combination of higher chlorine dioxide level and longer mixing time in air caused a larger increase in the asymptotic load than was expected by considering bleaching and mixing separately.

The technique of measuring structural relaxation of dough (2) provides the investigator with a tool that not only describes the relaxation phenomena in quantitative terms, but also enables one to postulate a model of the structure of dough and thus obtain a better understanding of the behavior of the system (5). With this technique the action of bromate (5) under various conditions (2,4,7) and of other agents (1) has been studied and measured in dough. In order to employ the technique for quality-control purposes, it is necessary to know the effect of flour bleaching on structural relaxation. Also it is necessary to choose a mixing time for the dough preparation. Consequently, the action of bleaching with chlorine dioxide and the effect of mixing time on dough structural relaxation were investigated.

Materials and Methods

The flour used in this study was commercially milled from North Dakota hard red spring wheat. The flour had a protein content of 11.9% and ash content of 0.41% on a 14% moisture basis.

Relaxation of dough was measured in a procedure using the unbleached flour and flour bleached with two concentrations of bleaching agent. The lower concentration consisted of 3.3 p.p.m. of chlorine dioxide and 200 p.p.m. benzoyl peroxide; the higher concentra-

[1] Manuscript received May 5, 1960. Presented at the 45th annual meeting, Chicago, Ill., May, 1960.
[2] Address: The Pillsbury Co., Minneapolis 2, Minnesota.

tion employed 5.5 p.p.m. of chlorine dioxide and 200 p.p.m. of benzoyl peroxide. These two concentrations are characteristic of the upper and lower limits of commercial treatment. The method of flour bleaching employed was essentially the acetic anhydride method described by Parker and Fortmann (9).

The relaxation procedure employed was essentially the one outlined by Dempster *et al.* (1,2). The dough was mixed in a standard farinograph mixer under room conditions. To 300 g. of flour, 2% of sodium chloride was added in solution plus enough water to make the absorption 60.8% on a 14% moisture basis. After 2.5 minutes of mixing, the dough had a consistency of 500 farinograph units. The dough was mixed for 2.5, 5, 10, or 20 minutes. A 0- or a 3-hour reaction time was given to the dough, which is the time between mixing in the farinograph and shaping with the extensigraph. Extensigrams were obtained after rest times ranging from 5 to 120 minutes.

From the extensigram the load at 5-cm. extension was measured. The extension recorded at 5 cm. on the chart was corrected for vertical movement of the dough holder. The extensigraph was calibrated at 2 extensigraph units per gram force.

Results and Discussion

Figures 1 and 2 show dough relaxation curves for unbleached flour at 0- and 3-hour reaction time with different mixing times. At a 1-hour reaction time the relaxation curves which are not shown fell in between the corresponding curves of 0- and 3-hour reaction time. Since the farinograph mixer was operated under room conditions, Figs. 1 and 2 show the total effect of the mechanical mixing action and oxygen incorporation into the dough.

Relaxation curve changes caused by varying mixing time are very slight for a zero reaction time as shown in Fig. 1. From 2.5 minutes to 10 minutes a slight increase can be noted in asymptotic load and relaxation constant for the hyperbolic curves. Increasing mixing time beyond 10 minutes to 20 minutes shifted the hyperbolic relaxation curve back down to the position of the 5-minute mixing time curve. The upward shift between 2.5- and 10-minute mixing is to be expected, assuming increasing oxygen incorporation with longer mixing time (3). The downward shift of the 20-minute mixing curve must be caused by mixing *per se*.

Figure 2 illustrates the effect of varying dough mixing time at a 3-hour reaction time. The upward shift with increasing mixing time was considerably greater for the curves at a 3-hour reaction time than it was at the 0-hour reaction time. At the 3-hour reaction time

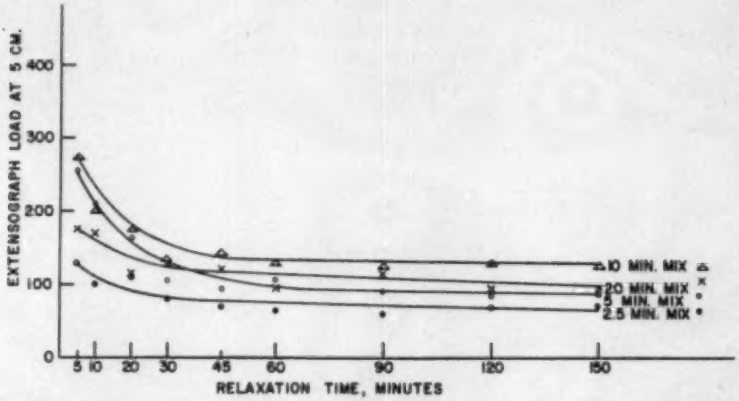

Fig. 1. Structural relaxation curves for dough mixed in air for 2.5, 5, 10, and 20 minutes in a farinograph mixer with a reaction time of 0 hours.

the increase of asymptotic load with longer mixing time held true up to 20 minutes of dough mixing in the farinograph mixer. At all the mixing times tested, the relaxation curves shifted upward with increasing reaction time. The upward shift probably means that the oxygen incorporated into the dough was beyond the level of 40% oxygen reported by Dempster *et al.* (3).

Relaxation curve parameters were calculated for the mixing time

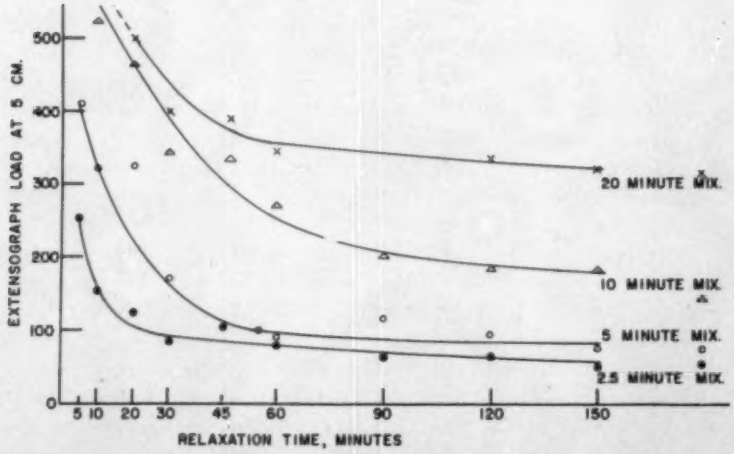

Fig. 2. Structural relaxation curves for dough mixed in air for 2.5, 5, 10, and 20 minutes in a farinograph mixer with a reaction time of 3 hours.

TABLE I

DOUGH STRUCTURAL RELAXATION PARAMETERS OBTAINED FOR UNBLEACHED AND BLEACHED FLOUR

Mixing Time	Reaction Time	Hard Red Spring Flour, Unbleached			Hard Red Spring Flour Bleached with 3.3 p.p.m. Chlorine Dioxide and 200 p.p.m. Benzoyl Peroxide			Hard Red Spring Flour Bleached with 5.5 p.p.m. Chlorine Dioxide and 200 p.p.m. Benzoyl Peroxide		
		Asymptotic Load	Relaxation Constant	Semiaxis Constant	Asymptotic Load	Relaxation Constant	Semiaxis Constant	Asymptotic Load	Relaxation Constant	Semiaxis Constant
minutes	*hours*									
2.5	0	75	2.0	2.0	89	2.9	2.4	115	2.4	2.2
2.5	3	79	1.1	1.5	106	3.7	2.7	141	6.4	3.6
10	0	111	2.6	2.3	138	2.0	2.0	144	2.0	2.0
10	3	146	10.4	4.6	225	15	5.5	300	19.4	6.2

data shown in Figs. 1 and 2. However, because of the considerable experimental error involved, the calculated relaxation parameters could be used only in a qualitative way to substantiate the observations already made from Fig. 1 and Fig. 2. The relaxation constant and asymptotic load increased with higher reaction time, this increase being larger for the longer mixing times.

The effects of flour bleaching on structural relaxation of doughs is shown in Table I for dough-mixing times of 2.5 and 10.0 minutes and reaction times of 0 and 3 hours. The asymptotic load, the relaxation constant, and the hyperbola semiaxis were calculated from regression equations.

Table I shows that physical changes of dough caused by commercial bleaching and a 3.3- to 5.5-p.p.m. change of concentration of chlorine dioxide can be measured by structural relaxation techniques. The asymptotic load increases with increasing concentration of bleaching agent for all four combinations of mixing time and reaction time. The bleaching effect, as measured by the increase in asymptotic load at 3-hour reaction time, is larger for the 10-minute mixing time than for the 2.5-minute mixing time.

The relaxation constant shows no pattern of change at 0-hour reaction time. It is probable that at 0-hour reaction time the relaxation constant which has a mean value of 2.3 is not affected by the concentration of bleaching agent. At 3-hour reaction time, however, the relaxation constant increases with increasing chlorine dioxide concentration. The increasing relaxation constant shows that the reaction caused by the bleaching is dependent on the concentration of bleaching agent. The reaction also is time-dependent, since it requires a reaction time, 3 hours in this case, to become measurable.

Table I shows that the effects of bleaching and mixing are not additive when measured by the relaxation constant. For example, the relaxation constant difference caused by the change of chlorine dioxide concentration is for the 2.5-minute mixing time 6.4−3.7 or 2.7 units. For 10-minute mixing, it is 19.4−15 or 4.4 units.

To obtain an additive index, the dough relaxation hyperbola semiaxis constants were calculated as proposed for the bromate reaction by Hlynka and Matsuo (8). The semiaxis difference between the doughs with the two chlorine dioxide concentrations was approximately the same regardless of mixing time. At 2.5-minute mixing, it was 0.9 units; at 10-minute mixing it was 0.7 units. Similarly, it was found that the semiaxis difference caused by the 2.5- to 10-minute difference in mixing time is the same, regardless of the bleach level used. The 2.5- to 10-minute difference in mixing time results in a semiaxis difference of 2.9, 2.8, and 2.6 for the three chlorine dioxide levels identified in Table I.

The approximately constant semiaxis constant difference means that bleaching effects were independent of effects produced by mixing under atmospheric conditions. Although relaxation rate differences as characterized by the hyperbola semiaxis were independent of mixing time, asymptotic load differences were not independent of mixing time. Thus, it was found that bleaching to the 5.5-p.p.m. level of chlorine dioxide caused an asymptotic load difference of 141−79= 62 extensigraph units at 2.5-minute mixing, while at 10-minute mixing the difference is $300 - 146 = 154$ units. Similarly, the asymptotic load increase due to the mixing time difference between 2.5 minutes and 10 minutes was 300−141=159 extensigraph units at the high chlorine dioxide concentration whereas it was 146−79=67 for the unbleached flour.

These results show that, using the farinograph mixer under room conditions, dough structural changes caused by bleaching may be measured as semiaxis differences at any mixing time. Asymptotic load changes due to bleaching will, however, be dependent on farinograph mixing time.

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PROTEIN GRANULES OF MAIZE ENDOSPERM CELLS[1]

DONALD N. DUVICK[2]

ABSTRACT

Cytoplasmic inclusions composed primarily of protein are found in the maize endosperm. They are roughly spherical, or soap-bubble shaped, numerous, and range in size from near the limit of resolution in the light microscope up to about 3 microns in diameter. They are largest and most numerous in the subaleurone cells, progressively decreasing in size from the outer to the inner cells of the endosperm. Their development, as followed under the light microscope, begins at 15–20 days after pollination in the region under the silk scar and spreads to the other portions of the endosperm. In a given region, enlargement of protein granules starts first in inner cells and then in cells progressively nearer the aleurone.

Histochemical tests, plus the positive correlation of period of maximum rate of growth of the protein granules with the reported period of maximum rate of increase in percent of zein during development of the endosperm and the positive correlation of the areas of greatest size of the granules with the reported areas of greatest zein concentration, suggest that protein granules are the major site of zein storage in the maize endosperm.

The growth and development in maize endosperm cells of a class of cytoplasmic inclusions termed "protein granules" has been described briefly in a previous publication (3). The present paper amplifies the descriptions given there and also relates the data to facts from other sources which deal with the protein components of the maize endosperm.

[1] Manuscript received May 5, 1960. Part of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at Washington University, St. Louis, Missouri. Presented at the 45th annual meeting, Chicago, Illinois, May 1960.
[2] Pioneer Hi-Bred Corn Company, Johnston, Iowa.

Materials and Methods

Detailed studies were made on two distinctively different open-pollinated varieties of maize: Longfellow New England Flint and Gourdseed Southern Dent. These two varieties are representative of the two types of corn which have been proposed as the ancestors of modern Corn Belt Dents (1). The kernels of Longfellow are nondented and have a layer of horny endosperm which completely surrounds the sides and crown of the kernel. The kernels of Gourdseed are long and deeply dented, so much so, in fact, that the crown of the kernel often is completely collapsed. Gourdseed kernels have little or no horny endosperm and are composed almost entirely of floury endosperm. The small amounts of horny endosperm that occasionally do occur are located on the periphery of the side and rear (abgerminal side) of the kernel, somewhat below the midsection. Some observations also were made on several other varieties of corn, most of which were Corn Belt inbred lines or Corn Belt hybrids.

Most of the material examined was grown at the Arboretum of the Missouri Botanical Garden at Gray Summit, Missouri, in 1949 and 1950. All kernels examined were taken from hand-pollinated ears, so that the exact time from pollination to harvest was known. At intervals from pollination to kernel maturity, kernels from freshly picked ears were fixed in Craf or Zirkle-Erliki fixative (8). Hand-cut sections of fresh kernels also were examined with the aid of dissection and compound microscopes. The fixed specimens were later embedded in paraffin and sectioned. It was necessary to soak the more mature specimens in water before sectioning. The sections were stained with Heidenhain's hematoxylin (8) or were treated in other ways, as described below. Some mature unfixed kernels were soaked in water for 24 hours and then sectioned with a freezing-microtome.

Various histochemical tests were made on fixed and unfixed kernel sections, including: 1) Millon reaction for tyrosine, Serra histochemical modification (14); 2) Millon reaction, Bensley histochemical modification (2); 3) xanthoproteic test, histochemical modification (14); 4) arginine reaction, Thomas's modification (15) of the Sakaguchi reaction; 5) tryptophan test, Romieu reaction (4); 6) tryptophan test, Voisinet-Fürth reaction (14); 7) iodine in potassium iodide (I/KI); and 8) Sudan III for lipids, Jackson procedure (4).

Micro-Kjeldahl determinations of protein nitrogen were performed on various endosperm regions of a few mature kernels. The method used was essentially that described by Peters and Van Slyke (13): the Arnold-Gunning digestion method with peroxide and titration of the distilled ammonia with boric acid.

Results

In all varieties of corn examined, inspection of the sections stained with Heidenhain's hematoxylin revealed that within a week after the first appearance of starch granules in the endosperm a second class of granules (called protein granules, for reasons given below) began to enlarge. These probably corresponded to the protein granules described in mature endosperms by Mottier (11) and Harz (6). Although exact counts could not be made, the granules did not seem to increase in number in any given part of the endosperm after their first appearance there, but their enlargement made them increasingly conspicuous in any given region, with the passage of time. The protein granules were at all times easily distinguished from starch grains. In mature endosperms stained with hematoxylin, a section through a horny endosperm cell had somewhat the appearance of a section through a box of white marbles (starch grains) in which buckshot (protein granules) has been used as packing between the marbles; the whole boxful is then filled with a transparent glue (clear viscous cytoplasm) which surrounds marbles and buckshot and makes the ensemble, when dry, a rigid conglomerate.[3]

By measuring the diameter of the protein granules in equivalent cells in successively older endosperms gathered at intervals of 5 to 10 days, one may determine growth curves for the protein granules of various regions of the endosperm. Figures 1 to 4 show such curves, smoothed in from scatter plots of several measurements, for Longfellow and for Gourdseed, 1950 collections. The curves show the protein granule growth in three regions of the endosperm, in two cells of each

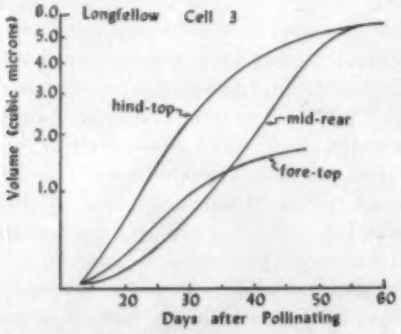

Fig. 1. Increase in volume of protein granules in cell 3 in three regions of Longfellow. Volume calculated on assumption that granules are spherical. See text for further explanation.

[3] Shown in illustrations in Duvick (3).

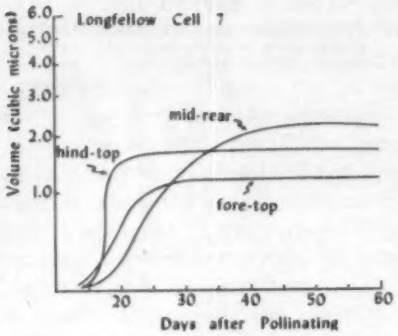

Fig. 2. Increase in volume of protein granules in cell 7 in three regions of Longfellow corn.

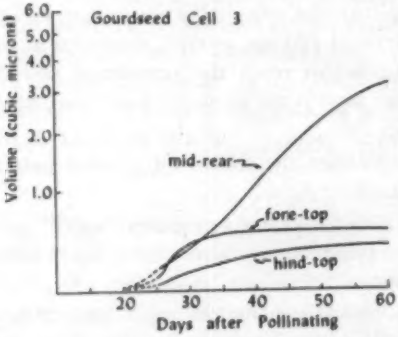

Fig. 3. Increase in volume of protein granules in cell 3 in three regions of Gourdseed corn.

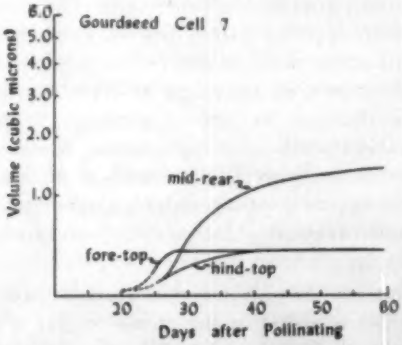

Fig. 4. Increase in volume of protein granules in cell 7 in three regions of Gourdseed corn.

TABLE I

MEAN DIAMETER OF PROTEIN GRANULES IN THREE ENDOSPERM REGIONS OF TWO VARIETIES OF MATURE CORN, 1950 CROP

VARIETY	REGION	CELL NUMBER			
		3	7	11	15
		μ	μ	μ	μ
Longfellow	Fore-top	1.6	1.6	1.3	1.0
	Hind-top	3.1	1.7	1.5	0.8
	Mid-rear	2.8	2.2	1.7	0.5
Gourdseed	Fore-top	1.0	0.7	0.4	..[a]
	Hind-top	0.8	0.7	..	..
	Mid-rear	1.9	1.7	1.4	1.1

[a] No. cell.

region. The regions are: a) "fore-top," on the crown just forward of the silk scar; b) "hind-top," on the crown well to the rear of the silk scar; c) "mid-rear," in the midsection of the kernel, opposite the germ. The cells are the third and the seventh, counting in from the aleurone. Counts were not begun until the peripheral cell divisions in each region had ceased; so it is reasonably certain that equivalent cells were observed.

The curves illustrate the following general patterns, noted in all materials examined:

1. Rapid growth of protein granules begins first in cells of the crown region and progressively moves down to cells of the basal portion of the endosperm.

2. In a given endosperm region, rapid growth of protein granules begins earlier and ends earlier in inner cells.

3. The final size of protein granules in the outer cells is greater than that of protein granules in inner cells. The average final size of the protein granules is progressively smaller, going from outer to inner cells of the endosperm, until in the center, somewhere in the floury endosperm region, protein granules, as such, cannot be identified. The progressive decrease in size of protein granules, going from peripheral to central cells in two different mature endosperms, is illustrated in detail in Table I. Note, by way of contrast, that starch grains in the endosperm are progressively larger, going from outer to inner cells, until about the 15th cell after which they, too, are progressively smaller (3).

4. An exception to the size progressions described for both protein and starch granules is found in the crown region of dent corns. The curves for Gourdseed, Figs. 3 and 4, illustrate an extreme example of this in the protein granules.

The final sizes of the protein granules are insignificant in the two crown regions of Gourdseed, as compared to the midsection of the same variety or to any of the three regions of Longfellow. In Gourdseed the crown region of the kernel dents; that is, the endosperm cells in the crown remain large and vacuolate until the kernel dries down at maturity, at which time they collapse. These "permanently" vacuolate cells have little or no development of either starch or protein granules. About the usual numbers of granules start development, but their growth stops abruptly at an early stage. This phenomenon is found in any dent corn; the deeper the dent, the greater the region of "permanently" vacuolate cells in the crown.

Longfellow, a nondent, illustrates the other extreme of development of the crown region. Here, all regions of the endosperm, including the crown, are composed of horny endosperm on the periphery and floury endosperm in the inner portions. Starch and protein granules of the crown as well as of the midsection are well developed. However, even in Longfellow, as well as in Gourdseed and all other varieties examined, cells of the extreme center portion of the endosperm remain vacuolate and collapse at maturity in the same fashion as do the crown region cells of the dents. Kernels which dent are merely those in which the center region of vacuolate cells extends up to, or nearly up to, the crown.

One further modification of the region of vacuolate cells should be noted: the centermost vacuolate cells usually rupture, leaving a fluid-filled hollow during the period of maximum endosperm expansion. The regions with ruptured cells, of course, also shrink at the time the kernel dries down.

Measurements of protein granules of the 1949 samples of Longfellow and Gourdseed produced a series of curves like those shown for 1950, except that the sampling period was shorter and the curves could not be extended as far as they were in 1950.

Proofs for the proteinaceous nature of the protein granules are as follows:

1. The granules (a) gave a positive test for tyrosine with the Millon-Serra reagent, either in freezing microtome sections or after Craf fixation and paraffin-embedding; (b) stained yellow with I/KI; (c) gave a negative reaction to the Sudan III test for lipids (fresh, freezing-microtome sections); and (d) were not birefringent in polarized light, even when starch grains nearly as small as they exhibited a clearly defined cross (3). The "clear" viscous cytoplasm, incidentally, gave a positive test with Millon and stained yellow with I/KI. It presumably is, therefore, proteinaceous.

Fig. 5. Portion of crown in section of mature Longfellow endosperm, stained with Millon, to show increasing depth of color in cells in progressing from center of endosperm (bottom of picture) to aleurone layer (top of picture). White spheres are starch grains. Unfixed, freezing-microtome section. 70×.

2. When whole endosperm sections were examined with the unaided eye or under the dissecting microscope, the regions which were stained most deeply by the Millon-Serra reaction (Fig. 5), by the Bensley modification of the Millon, by I/KI (yellow), and by the xanthoproteic reaction corresponded closely to those regions in which the protein granules were known to be largest and appeared to be most numerous.

3. Micro-Kjeldahl nitrogen determinations of various endosperm regions of two mature kernels of Longfellow, shown in the table below, indicated that the regions which had the largest sizes and apparent numbers of protein granules (the subaleurone regions) also

Region	*Cell No.*	*Protein, at 14% Moisture*[a]
		%
Aleurone	1	36
Outer horny endosperm	2–7	22
Inner horny endosperm	8–14	9

[a] Mean of two determinations on each of two kernels.

had the largest percentages of protein. This minuscule experiment has been confirmed on a larger scale by Hinton (7), who has shown in another variety of flint corn that the protein percentage of the horny endosperm is greater in the subaleurone cells than it is in those horny endosperm cells nearer the center of the endosperm.

There is some evidence that the protein granules may be composed largely, or only, of the complex of proteins presently designated as zein. Evidence for this statement is given in the following numbered paragraphs.

1. The protein granules give a negative test for arginine, although aleurone cells give a strong positive test and the clear cytoplasm of the endosperm cells also gives a definite, although not a strong, test for arginine. Zein contains little arginine as compared to the glutelins which, with zein, comprise the major storage proteins of the endosperm.

2. When freezing-microtome sections of the horny endosperm region of kernels of various types of corn were shaken in 0.2% sodium hydroxide for 30 minutes or left to stand for as little as 4 hours, the starch grains and protein granules of most cells fell from the clear viscous cytoplasm in which they had been encased, and the clear cytoplasm itself largely disintegrated. In an early stage, the protein granules were thickly clustered over the surface of the starch grains. When equivalent sections were shaken for 30 minutes or left standing up to 120 hours in 70% ethyl alcohol, no change could be seen at all, either before or after staining with iodine or with Millon reagent. When sections were kept up to 120 hours in a 1:3 mixture of 0.2% sodium hydroxide and 70% ethanol, both clear cytoplasm and protein granules disappeared from most cells. This treatment was not greatly different in apparent effect, however, from that with alkali alone. A 5% sodium chloride solution had no visible effect on either clear cytoplasm or protein granules after as long as 120 hours. These differential solubility reactions are in agreement with the supposition that most of the clear cytoplasm is of the alkali-soluble glutelin type of protein and that, because it surrounds the alcohol-soluble protein granules, it protects them from dissolution by alcohol solution. In routine zein extraction alcohol may be used as the first solvent, but the grain is first ground and the grinding must reveal surfaces that are protected in the intact cell.

In a series of experiments to determine the effects of (a) sequence of treatments and (b) pretreatments, Nagy *et al.* (12) found that, when ground grain was extracted directly with ethanol, it was necessary to add sodium acetate to get maximum extraction of zein. Maximum amounts of zein could also be extracted, however, when the grain was first treated with weak alkali and then extracted with ethanol, without sodium acetate; but, if the grain in alkali was neutralized and then treated with 5% salt, which precipitated both glutelins and zein, the zein then could be maximally extracted only by again first adding weak alkali. All of these results agree with the supposition that the

zein may be in the protein granules, surrounded by clear cytoplasm made up largely of alkali-soluble proteins. Any treatment which first peptizes the clear cytoplasm will then expose a maximum number of the granules to the action of ethanol. Nagy *et al.* (12), in stating that "a part of the zein may be adsorbed on the glutenin [sic] (or on other material) as it is synthesized by the plant," presumably envisaged a more intimate union between the two classes of proteins than is supposed in the hypothesis presently advanced.

3. The time of maximum increase in size for most of the protein granules seems to coincide with the time of greatest increase in amount of zein in the endosperm. Watson (16) and Zeleny (18) have stated that zein deposition in the endosperm takes place largely in the latter portion of the endosperm maturation period. Watson's data show that the highest rate of zein deposition occurs during approximately 14 to 35 days after pollination and continues at an appreciable rate until complete grain maturation. Figures 1–4 show that maximum rates of increase in the size of protein granules in all of the endosperm regions of Longfellow began no sooner than about 2 to 3 weeks after pollination and that for Gourdseed (a much later-maturing variety of corn) maximum rates were not reached before about 4 weeks after pollination. The area of major deposition of protein granules in Gourdseed includes that represented by cells 3 and 7 of the mid-rear region, and here it is apparent that much of the size increase occurred as late as 50 days after pollination. Recent data given by Mertz (10) indicate, however, that the zein percentage of endosperm protein does not increase with time; so it may be that a re-examination of zein-accumulation data is needed.

4. The data of Hamilton *et al.* (5) indicate that the percentage of zein in horny endosperm is nearly twice that for zein in floury endosperm. As noted above, the size and apparent numbers of protein granules are progressively greater, from inner cells, in the floury endosperm, to outer cells, in the horny endosperm. The presence of identifiable protein granules apparently does not, *per se*, determine whether endosperm is horny or floury; rather, there seems to be a threshold point at which the proportion of viscous protein (clear cytoplasm) to granular inclusions (starch grains and protein granules) becomes such that the viscous protein shatters during the time the kernel is drying down. Cells in immature endosperms have a relatively high percentage of water, and the viscous protein in all cells is plastic enough and plentiful enough to encase all granules. As the endosperm dries down during the maturation process, the viscous protein loses volume and elasticity because of water loss; it shrinks and sometimes

TABLE II

MEAN DIAMETER OF PROTEIN GRANULES IN ENDOSPERM OF TWO VARIETIES OF CORN AT TWO LEVELS OF PROTEIN CONTENT

	G-94		Illinois High Protein	
	7.5% Protein	10.7% Protein	13.3% Protein	20.6% Protein
	μ	μ	μ	μ
Cell 3	0.9	1.2*	1.3	1.8**
Cell 7	0.5	1.0**	1.0	1.6*

becomes insufficient in volume to encase all granules completely; in such cases, it ruptures. This process can be followed under the microscope. The cells in which the viscous protein has been thus shattered become the floury endosperm cells; they are filled with refractive air spaces that appear white (6). As a general rule, these cells have rounded, loosely packed starch grains and small protein granules which are insufficient to pack the large interstices between starch grains. In subaleurone cells, starch grains are rounded, but interstices are filled with tightly packed protein granules, and the proportion of viscous cytoplasm to granules seems to be somewhat higher. In the horny endosperm cells farther from the periphery, the starch grains are compressed so much that the interstices are relatively small.

5. In samples of seed of varying protein percentage,[4] kernels of the hybrid G-94, grown with ample amounts of nitrogen fertilizer and with 10.7% protein in the whole grain, have larger protein granules in the mid-rear section of the horny endosperm than do kernels of the same hybrid grown without nitrogen fertilizer and which have only 7.5% protein. Kernels of Illinois High Protein, grown with ample nitrogen fertilizer and having 20.6% protein, had larger protein granules in the mid-rear section of the horny endosperm than did kernels of Illinois High Protein grown without nitrogen fertilizer and which had 13.3% protein (Table II). Numerous investigations have shown that when protein percentage is increased as a result of nitrogen fertilization, the principal increase is in the zein fraction (5).

6. The Voisinet-Fürth test for tryptophan, when used on sectioned kernels, did not show a gradation in depth of color which precisely correlated with the change in size of the protein granules, as did the Millon and xanthoproteic reaction, both of which are specific for tyrosine in proteins. Rather, the tryptophan test produced a pink color evenly over the whole endosperm except for a slight intensification of color in the outermost two or three cells. In these outer cells, it appears that the proportion of clear cytoplasm to granular inclusions is higher

[4] Kindly supplied by E. B. Earley.

than in any other part of the endosperm. The Romieu reaction for tryptophan, although used only on split rather than sectioned kernels, gave the same pattern of coloration as did the Voisinet-Fürth test on sectioned kernels. Tyrosine percentage is about the same in zein as in the glutelins; whereas it is a fact of long-standing nutritional knowledge that zein has almost no tryptophan (0.1%) but that glutelins have, by contrast, much more (about 1%) (9). Therefore, the even coloration over the endosperm indicates that the granules did not contain glutelins in proportion to their size and apparent numbers. The reactions of the tryptophan tests were too weak to allow identification of color in any specific cytoplasmic inclusions, at the high magnifications necessary for such inspection.

Discussion

Although the data given in this paper do not prove beyond all reasonable doubt the exact locations of the intracellular sites of deposition of zein and of glutelins, the information is sufficient to make further investigation seem worth while.

Implications of the hypothesis, both to those interested in methods of separating maize endosperm fractions and to breeders interested in changing the proportions and amounts of endosperm proteins, are obvious. It seems probable that study of endosperm cells of the other cereal grains will show similar cytoplasmic structures and similar relationships between inclusions and protein types. Watson *et al.* (17), for instance, have described protein granules in sorghum endosperm and have shown in photographs the similarity between these structures in corn and sorghum.

It may be pertinent to note that the earlier literature (e.g. Mottier, 11) has abundant descriptions of protein granules in various plant seeds (including maize), usually in endosperms of monocotyledonous plants and in cotyledons of dicotyledonous plants. It seems reasonable to suppose that such specialized storage organelles would each store a fairly distinct type or group of related types of protein. Thus, even though zein as extracted according to the old method of differential solubilities may not be a single molecule, a family of zeins might, nevertheless, be associated in the same cytoplasmic organelle.

It might be speculated further that in maize endosperm, the protein granules, storing mostly zein, are the variable which can most easily change to store more or less protein, depending on the genotype and the environment. The viscous cytoplasm, by comparison, probably forming its major storage proteins prior to zein accumulation in any given cell, may be a relatively stable component, quantitatively, as

influenced by either environment or heredity. It should be emphasized that these statements are, at present, conjectural.

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CONVERSION OF CONSTANT-FLOUR FARINOGRAPH ABSORPTION TO CONSTANT-DOUGH BASIS[1]

I. HLYNKA[2], F. D. KUZINA[2], AND W. C. SHUEY[3]

ABSTRACT

The relation between the amount of water used in preparing farinograms by the constant-dough method (W_{CD}) and the water in the constant-flour method (W_{CF}) is formulated as

$$W_{CD} = W_{CF}\ (80/D_{CF}) + K\ (80 - D_{CF})$$

where D_{CF} denotes the dough weight in the constant-flour method, 80 is the dough weight in the constant-dough weight method (small bowl), and K is a constant. An identical equation may be written for the large bowl with 480 replacing 80 as the constant weight of dough.

The proportionality constant K was evaluated experimentally and was found to be 0.05. The same constant was found to apply for the large and the small bowl and for different classes of flour.

With this constant and equation, farinograph absorptions obtained by the constant-flour method can be readily converted to the constant-dough basis.

Cereal Laboratory Methods (1) describes two procedures for the use of the farinograph. In one method, a constant amount of flour is used with sufficient water to obtain a curve centered about the 500-unit line at its maximum; the weight of the dough in the mixing bowl varies from flour to flour. In the second method, the total weight of dough is kept constant, whereas the ratio of flour to water is varied. At 60% absorption and at 14% moisture the two methods coincide, but, at lower or higher absorptions, considerable differences between the two methods have been reported (2,3).

This paper presents data and discusses the conversion of farinograph absorption obtained by the constant-flour method to predict quantitatively the results on a constant-dough basis.

Basis of Relationship between the Two Methods. The relation between the amount of water used in the constant-dough method (W_{CD}) and the water in the constant-flour method (W_{CF}) may be formulated in mathematical language as follows:

$$W_{CD} = W_{CF}\ (80/D_{CF}) + K\ (80 - D_{CF}) \qquad (1)$$

where D_{CF} denotes the dough weight in the constant-flour method, 80 is the dough weight in the constant-dough weight method (small bowl), and K is a constant. This means that the water used in the

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constant-dough method is equal to the water used in the constant-flour method, both calculated to the same basis of 80 g., plus a correction factor which depends on the difference in the amount of dough between the two methods.

An identical equation may be written for the large bowl with 480 replacing 80 as the constant weight of dough.

The proportionality factor K in the above equation can be readily evaluated experimentally. A series of precise data by the two methods on representative flours is necessary; the constant so obtained may then be used to translate data obtained by the constant-flour method to the constant-dough method basis.

Materials and Methods

In all, 64 flours were used in this study. These flours represented hard red spring (HRS), hard red winter (HRW), soft red winter (SRW), and soft white (SWW) wheats. Most flours were of American and some of Canadian origin. The farinograph absorptions ranged from approximately 50 to 70%.

The work was done in two laboratories using three farinographs, three small bowls, and three large bowls.

The constant-flour weight and the constant-dough weight methods were followed as outlined in *Cereal Laboratory Methods* (1). It was advantageous to record the results as the amount of water added to the flour instead of on the usual percentage basis. The percent-flour basis and the 14% moisture basis are best regarded as special cases of the more general treatment adopted here. A high degree of precision in the data was aimed at, especially where the differences between the two methods were small.

Results

Evaluation of the Proportionality Constant K. The first step in processing of the experimental results was to evaluate the constant K in equation 1. This was done by substituting experimental values for the water required by each method, together with appropriate dough-weight data in equation 1, and calculating K for each flour.

A careful study of the results led to the following conclusions. The same factor was obtained for each of the different classes of flour: hard red spring, hard winter, and soft. Moreover, the same factor was obtained for the small and the large bowl, provided that the data were expressed as water added to the flour used.

An over-all proportionality constant K of 0.05 was obtained from the mean data. What this constant means is that a bulk of each gram

of dough above or below 80 or 480 g. has the same effect on the farinograph chart as 0.05 ml. water less or more, respectively. The precision of this value for K is illustrated in the next section by applying it to one set of data for the small bowl and to one set for the large bowl.

Comparison of Experimental and Predicted Values for the Constant-Dough Absorption. In order to illustrate the type of results that can be obtained with the proposed proportionality constant, as well as to indicate the precision of the results, selected data are summarized in Tables I and II. The data on the small bowl were obtained by the Grain Research Laboratory and those for the large bowl by General Mills' laboratory.

TABLE I

EXPERIMENTAL AND PREDICTED CONSTANT-DOUGH WEIGHT ABSORPTIONS IN TERMS OF WATER ADDED TO FLOUR USED – SMALL BOWL

Flour Type	Flour Moisture	Weight of Flour (14% m.b.)	W_{CF} Experimental	D_{CF}	W_{CD} Experimental	W_{CD} Predicted
	%	g	ml	g	ml	ml
HRS	11.8	48.75	35.60	84.35	33.50	33.54
HRS	13.8	49.90	33.70	83.60	32.05	32.07
HRS	13.4	49.65	33.40	83.05	32.00	32.02
HRS	12.8	49.30	33.30	82.60	32.05	32.12
HRS	13.2	49.55	31.95	81.50	31.25	31.28
HRS	14.0	50.00	31.70	81.70	30.95	30.96
HRW	13.5	49.70	31.55	81.25	31.05	31.00
HRW	13.3	49.60	31.10	80.70	30.70	30.80
SRW	11.0	48.30	28.55	76.85	29.90	29.88
SRW	11.7	48.70	27.80	76.50	29.30	29.24
SWW	12.8	49.30	26.25	75.55	28.00	28.02

TABLE II

EXPERIMENTAL AND PREDICTED CONSTANT-DOUGH WEIGHT ABSORPTIONS IN TERMS OF WATER ADDED TO FLOUR USED – LARGE BOWL

Flour Type	Flour Moisture	Weight of Flour (14% m.b.)	W_{CF} Experimental	D_{CF}	W_{CD} Experimental	W_{CD} Predicted
	%	g	ml	g	ml	ml
HRS	11.7	292.2	211.2	503.4	199.9	200.2
HRS	10.4	288.0	208.2	496.2	199.9	200.6
HRS	12.8	295.9	191.3	487.2	188.0	188.1
HRW	10.4	288.0	192.0	480.0	192.0	192.0
HRW	12.1	293.5	190.2	483.7	188.5	188.6
HRW	13.0	296.6	189.1	485.7	186.7	186.6
HRW	13.3	297.6	185.0	482.6	183.4	183.9
SRW	12.0	293.2	171.5	464.7	177.6	177.9
SRW	10.8	289.2	170.8	460.0	178.6	179.2
SRW	11.6	291.9	165.2	457.1	175.2	174.6
SWW	11.8	292.5	161.0	453.5	171.7	171.7

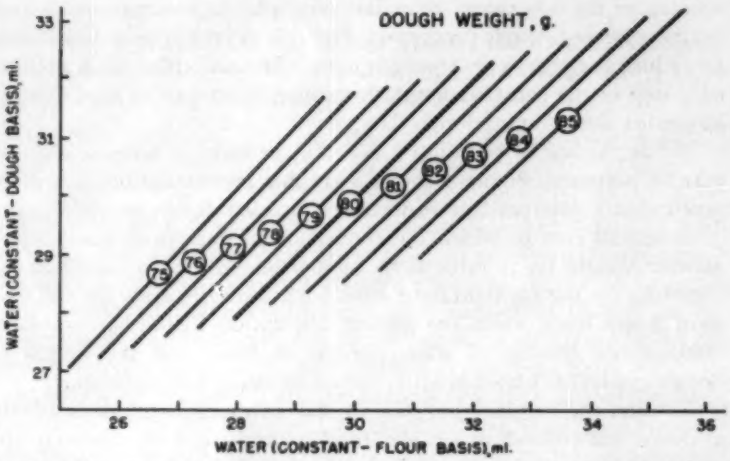

Fig. 1. A conversion graph to obtain constant-dough data from constant-flour farinograph data.

Table I summarizes the data for the small bowl. The first column shows the range of flours used in the study. The next four columns provide data on the amounts of flour, water, and the resulting dough in each instance. The last two columns show a comparison of the amount of water required in the test, determined experimentally with the calculated or the predicted value. The calculated values were obtained by substituting the appropriate values from the table into equation 1, using a value of 0.05 for the proportionality constant. Table II shows corresponding data for the large bowl.

A comparison of the experimental and predicted values for the constant-dough method shows a high order of precision. In the small-bowl data, the largest difference is 0.1 ml. water. In the large-bowl data, the largest difference is 0.7 ml. water. In terms of absorption percent, the mean data indicate the differences to be approximately 0.1%. If it is considered that both the constant-flour and constant-dough data contain an experimental error, it must be concluded that the value of K is highly satisfactory.

General Application of the Method of Conversion of Constant-Dough and Constant-Flour Farinograph Data. It is suggested that the method of conversion of constant-flour data to the constant-dough basis should be of value in three different areas. These will be noted briefly.

The titration method in the constant-flour procedure is somewhat

simpler in the laboratory than the successive-approximation method in the constant-dough procedure. For this reason, the constant-flour procedure together with equation 1 may be used either as a preliminary step in the constant-dough procedure, or it may be used directly to predict the constant-dough data.

While the use of equation 1 is to be preferred, a table or a graph may be prepared. Figure 1 shows a graphic representation. It will be noted that a different line is necessary for each dough weight.

A second area in which the conversion of data from one basis to another should be of value is in comparison of data reported in the literature on the constant-flour basis with those reported on the constant-dough basis. From the percent absorption in the constant-flour method, the amount of water, weight of flour, and the weight of dough can be calculated readily, preceding the use of equation 1.

Finally, the original purpose of this investigation was to obtain a closer understanding of the fundamental relation between the constant-flour and constant-dough farinograph procedures. The data presented in this paper should contribute to a more thorough understanding and a better appreciation of the two procedures.

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NOTE ON VARIATIONS IN RESIDUAL BRAN IN INDIVIDUAL RICE GRAINS FROM BATCHES POLISHED TO DIFFERENT DEGREES[1]

A. N. Srirangarajan, H. S. R. Desikachar, K. Indiramma,[2] and A. N. Sankaran[2]

The removal of bran from rice in milling is a chance process, and variations in the residual bran from grain to grain are, therefore, to be expected. Examination of individual grains in the white varieties of rice grains after staining the bran with methylene blue (1) or in varieties with a colored pericarp brings out these differences sharply. A quantitative evaluation of this variation is described here.

A variety of rice with a red pericarp (Kar-S. 1043) was chosen for study since the bran color could be extracted easily and measured in individual grains. Samples (2–9 in Fig. 1) polished to different degrees

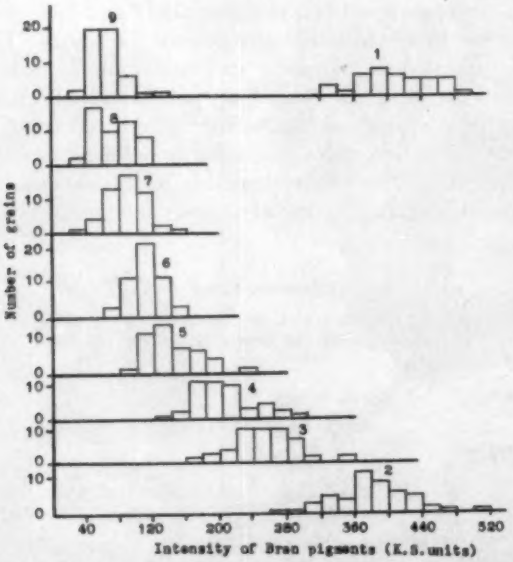

Fig. 1. Histograms for intensity of residual bran pigment in individual grains from batches polished to different degrees. 1, Hand-dehusked rice; 2, rice dehusked in mechanical sheller; 3–9, samples milled to different degrees in a polishing cone. (Analytical data for samples 2–9 are given in text. Data for sample 1 could not be obtained because the sample was lost.)

[1] Contribution from Central Food Technological Research Institute, Mysore, India.
[2] Division of Information and Statistics.

in a commercial polishing cone contained, respectively, in increasing order of polishing 2.59, 2.19, 2.01, 1.44, 1.08, 0.95, 0.89, and 0.86 mg. per g. of phosphorus and 3.06, 2.04, 1.97, 1.19, 0.96, 0.89, 0.71, and 0.58 γ per g. of thiamine. A small sample was dehusked by hand for comparison.

Fifty grains were selected at random from each lot of polished rice and the color of the bran was extracted from each grain by boiling it for 20 minutes with 3 ml. of 2% sodium bicarbonate solution. It was made up again to 3 ml., filtered, and color measured in a photoelectric colorimeter using micro attachment with No. 42 filter. To allow for differences in weight of individual grains, the color intensities were recalculated to a standard weight of 25 mg. The data are presented as frequency diagrams for the distribution of bran color intensity of rice grains at various stages of polishing (Fig. 1).

At each stage of polishing, there was variation in the color intensity of bran from the grains, which indicated the disparity in the degree of bran removal suffered by individual grains. It is interesting to note that even in unpolished rice (samples 1 and 2) there was large variation in the bran color intensity among the grains. This can be ascribed to the inherent variation in pigment deposition in grains. The variances at different stages of polishing were subjected to Bartlett's test and were found to be significantly different ($P<0.001$), indicating that there was a real variation in color intensity caused by differences in milling apart from those due to natural causes. The rate of bran removal was faster in the early stages of polishing than in the final stages.

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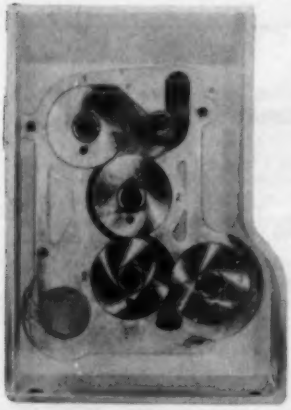

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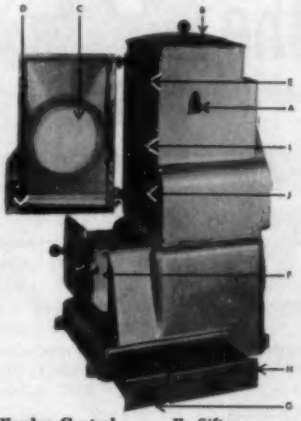

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